Rapid Quantification of Viable Campylobacter from Chicken Carcasses, Using Real-time PCR and PropidiumMonoazide Treatment, as a Tool for Quantitative Risk Assessment

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POSTER ABSTRACTS

P1-01 Prevalence of Spore-forming Bacteria in Food Using a Multiparametric PCR-based Tool

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Introduction: Sporeforming bacteria are ubiquitous in the environment and exhibit a wide range of diversity leading to their natural prevalence in foodstuffs.

Purpose: The state-of-the-art of sporeformers' prevalence in ingredients and food was investigated using a multiparametric PCR-based tool that enables simultaneous detection and identification of various genera and species mostly encountered in food, i.e., *Alicyclobacillus, Anoxybacillus flavithermus, Bacillus, Bacillus cereus* group, *Bacillus licheniformis, Bacillus pumilus, Bacillus sporothermodurans, Bacillus subtilis, Brevibacillus laterosporus, Clostridium, Geobacillus stearothermophilus, Moorella* and *Paenibacillus* species.

Methods: A total of 90 food products, with or without visible trace of spoilage were analyzed, i.e., 30 egg-based products, 30 milk and dairy products and 30 canned food and ingredients. Detection and identification of targeted species were performed using GeneDisc® Plate. In addition, spore enumeration and isolate 16S rDNA sequencing was used to validate PCR-based tool identification and identify other possible contaminants.

Results: Good correlations between the microbiology and molecular-based approach underline the great performance of this multi-detection tool. Most samples contained one or several of the targeted genera and species. For all three tested food categories, 30 to 40% of products were contaminated with both *Bacillus* and *Clostridium*. The percentage of contaminations associated with *Clostridium* or *Bacillus* represented 100% in raw materials, 72% in dehydrated ingredients and 80% in processed foods. In the last two product types, additional thermophilic contaminants were identified (*Anoxybacillus flavithermus, Geobacillus* spp., *Thermoanaerobacterium* spp. and *Moorella* spp.).

Significance: The results suggest that selection, and therefore the observed (re)-emergence of unexpected sporeformer contaminants in food might be favored by the use of given food ingredients and food processing technologies.

P1-02 Prevalence and Heat Resistance of Thermophilic Spore-forming Bacteria Contaminating Milk Powder

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Introduction: Spore-forming bacteria are ubiquitous in the environment and exhibit a wide range of diversity leading to their natural prevalence in foodstuffs. More particularly, thermophilic spore-formers such as *Anoxybacillus flavithermus* is not detected in raw milk but is frequently encountered in spray-dried milk powder.

Purpose: The aim of this study was to investigate the prevalence of spore-formers in milk and milk powder samples and determine wet heat resistances of spores naturally found to contaminate milk powders.

Methods: Several raw, pasteurized and processed milk samples were analyzed together with milk powder samples. Detection and identification of spore-forming bacteria was performed using GeneDisc® Plate multi-parametric tool. In addition, spore enumeration and isolate 16S rDNA sequencing was used to validate PCR-based tool identification and identify other possible contaminants. Mixed, naturally-encountered spores were collected from milk powder by filtration and wet heat resistances were evaluated on mixed spore suspensions. Milk powder major contaminants, i.e., *Geobacillus* and *Anoxybacillus*, were used to produce spores, for which heat resistance was determined.

Results: Native spores concentrated from raw milk samples show low spore counts with high spore-former diversity while processed and milk powders show lower diversity. *Bacillus coagulans, Bacillus licheniformis,* and *Ureibacillus thermosphaericus* were recovered in raw milk and processed milk. Species found in milk powders were mainly composed of thermophilic species such as *Geobacillus stearothermophilus, Anoxybacillus flavithermus* and *Ureibacillus* spp. Heat resistance of dominant spore-former contaminants in milk powder enable spore survival to industrial processes.

Significance: These results suggest that selection, and therefore the observed (re)-emergence of unexpected spore-former contaminants in food might be favored by the use of given food processing technologies.

P1-03 Modeling the Impact of Temperature and Water Activity on the Growth of *Aspergillus flavus, Cladosporium cladosporoïdes, Eurotium herbariorum, Penicillium chrysogenum* and *Wallemia sebi*

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Introduction: Development of molds such as *Aspergillus flavus, Cladosporium cladosporoïdes, Eurotium herbariorum, Penicillium chrysogenum* and *Wallemia sebi* is responsible for various food spoilage.

Purpose: The aim of this work is to develop a predictive model to simulate mold growth in bakery foodstuffs taking into account the impact of temperature and water activity (a_w) on fungal development.

Methods: Potato Dextrose Agar medium with a wide range of a_w (from 0.8 to 0.99) was performed after glycerol adjustment. Temperature was studied in the range of 10 to 40 °C. Two parameters of fungal development were taken into account, i.e., growth rate, expressed as the increase of colony diameter per day, and the lag time or apparition time of molds. The Rosso model (1993) was used to describe the influence of temperature and a_w on growth rate. Cardinal values were determined for water activity ($a_w$min, $a_w$opt and $a_w$max) and temperature (T_{min}, T_{opt}, T_{max}) for each studied mold.

Results: Molds tested presented different optimal growth temperatures, i.e., *Wallemia sebi* had the lowest T_{opt} (21 °C) and *Aspergillus flavus* the highest (31 °C). Great differences were also observed for the minimum a_w values for growth ($a_w$min): *Eurotium herbariorum* had the lowest $a_w$min value (0.70) and *Wallemia sebi* had the highest $a_w$min value (0.74). Great correlations were observed between this study and published cardinal values for temperature. Except for *Wallemia* and *Eurotium*, for which it was shown lower $a_w$min value than published data. Using the developed model, optimal growth rates (μ_{opt}) of the five species of mold were also evaluated on bakery products during storage at 25 °C (with various formulations and various a_w). No impact of the formulation on the growth rate was observed.

Significance: This study shows that predictive models developed for the simulation of bacterial growth may be used to describe the development of molds on bakery product matrices.

P1-04 Tolerance of *Bacillus cereus* against Low-dose γ-Irradiation and Cold Temperature Storage

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Introduction: Gamma irradiation has been highly recommended as a sanitary treatment to ensure the safety of food and food products from microbiological contamination. *Bacillus cereus* has been implicated in foodborne outbreaks. The majority of bacteria barely withstand γ-irradiation up to 5 kGy and cold temperature treatment.

Purpose: The objective of this study is to investigate the level of tolerance of *Bacillus cereus* strains KCCM 40138 to the combined treatment of low-dose γ-irradiation and storage at 0°C for 24 h after irradiation.

Methods: B. cereus at a concentration of 1.5 x 10^7 CFU/ml were exposed to the γ-irradiation dose of 0, 1, 1.5, 3 and 5 kGy and immediately stored at 0°C for 24 h. The optical density and the antimicrobial sensitivity profile were measured to compare the changes in between before and after the radiation treatment. PCR analysis that targets *B. cereus gyrB* gene region was carried out to observe the effect of gamma-irradiation on genomic DNA. In addition, electron microscopy was employed to observe the morphological differences among bacteria cells. Furthermore, the reduction in bacterial load caused by the irradiation was determined for each radiation dosage used.

Results: The results indicated that *B. cereus* could survive the γ-irradiation up to 5 kGy in spite of the reduction in number by more than 70%. The survived *B. cereus* retained their important features. Since *B. cereus* could be positively identified by PCR analysis, it can be assumed that the γ-irradiation did not affect the selected portion of genomic DNA which was used for identification in all doses used. There was no significant change observed in optical densities and antimicrobial sensitivity profile, before and after irradiation. Since low-dose γ-irradiation below 3 kGy is insufficient to achieve the decontamination of *B. cereus* from food substances, higher dosage such as more than 5 kGy is needed to achieve proper decontamination. It is also suggested that since decontamination does not occur uniformly to all bacteria, different doses of γ-irradiation may be required for different bacteria.

Significance: Uniform decontamination of food and food products from bacteria by γ-irradiation is still a challenging debate to which this research makes a contribution.

P1-05 Influence of Water Activity and Ascospore Age on the Growth of *Byssochlamys nivea* in Papaya Juice

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Introduction: So far, very little has been published on the effect of environmental factors, as ascospore age ("A") and water activity ("a_w"), on the growth of heat-resistant mold in commercial products. The interest of researchers for studying *Byssochlamys nivea* ascospores is that this mold can survive commercial pasteurization, diminishing the shelf life of these products.

Purpose: Since the time that the ascospores remain in the environment and the effect that it can cause on mold growth are still unknown, the aim of this research was to evaluate the influence of "a_w" from 0.90 to 0.99 and the "A" from 30 to 90 days in vitro incubation on the growth of *B. nivea* in papaya juice.

Methods: The sporulation of the strain was performed in MEA and incubated at different "A" at 30°C. Papaya juice was used as growth media and adjusted to different "a_w" values and the mold growth was evaluated by the measurement of the radial growth. The suspension of *B. nivea* was previously activated and incubated in polyethylene terephthalate bottles. The growth parameters were determined by the fit of Modified Gompertz (MG) and Logistic (L) models to experimental data to obtain growth parameters maximum specific growth rate (μmax); colony diameter (A) and phase lag duration (λ).

Results: Both models MG and L adjusted well to the experimental data, but the MG model showed slightly higher lag phase adjust. The result showed that "A" did not influence the *B. nivea* growth; however, "a_w" was statistically significant ($P < 0.05$) to the growth parameters λ and μmax. The minimum values of λ and μmax, calculated by the MG model, were 9.6 h and 0.020 mm/h, while the maximum values were 272 h and 0.046 mm/h, respectively.

Significance: The results acquired are relevant for the prevention of spoilage of fruit juices on the studied range of water activity, diminishing industry losses.

P1-06 Heat Inactivation of *Leuconostoc* spp. on Cooked, Vacuum-packaged Sausage

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Introduction: Microbial spoilage in Vienna sausages and other processed meats is frequently caused by *Leuconostoc* spp. Microorganisms can contaminate sausages after the cooking step and cause the premature spoilage of sausage. A post-packaged heat treatment could minimize the sausage microbial load and avoid its spoilage during storage, distribution and food commercialization.

Purpose: Evaluate the heat inactivation of *Leuconostoc mesenteroides* and *L. lactis* strains on cooked, vacuum-packaged sausage.

Methods: L. mesenteroides and *L. lactis* strains (rifampicin resistant) isolated from the sausage production environment were inoculated on cooked turkey sausage (10^8 CFU/unit). Six sausages (3 x 2 units) were vacuum-packaged and exposed to heat treatment (70-80°C/30-50 min). Sausage packages were chilled in ice (30 min) and survivors cells were quantified in sausages located in the center of the package. MRS agar containing 100 ppm of rifampicin were used for microbial count. Central composition design was applied.

Results: Statistical analysis showed as a significant factor the time of heat exposition ($P < 0.05$). The average log reductions (all temperatures) in the *L. mesenteroides* population after 50, 40 and 30 min of heat treatment were 4.5. 1.72 and 1.03, respectively. The corresponding values for *L. lactis* were 4.16, 1.9 and 1.4 log of CFU. Only the sausages exposed to 80°C/50 min showed a visual loss of firmness.

Significance: Heat treatment of sausage at 70°C reduces the *Leuconostoc* spp. population and could improve the food shelf life.

P1-07 Assessment of the Level of Microbiological Contamination of Unpackaged Spices Commercially Available in a Market in Ankara, Turkey

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Introduction: Spices are widely used throughout the world in food preparation and processing for their flavor and aromatic properties. Spices can become contaminated due to the nature of their source and production conditions. The level of microbiological contamination could potentially be increased if spices are sold unpackaged.

Purpose: To determine the level of microbiological contamination of 18 types of spices sold unpackaged and in bulk in a market in Ankara, Turkey.

Methods: Eighteen samples were collected from three vendors (six samples/vendor) located in an open-air market in the Ulus region of Ankara. Samples were analyzed for mesophilic aerobic plate counts (APC) and generic *Escherichia coli*. APC were determined using Tryptone Glucose Extract (TGE) agar and evidence of the presence of generic *E. coli* was determined using ENDO™ agar.

Results: APC ranged from <10 CFU/g to 2.3 x 10^3 CFU/g for all samples. Individual samples that were most highly contaminated were mixed-spices samples (2.3 x 10^3 CFU/g), curry powder (2.2 x 10^3 CFU/g), red pepper powder (Capsicum annuum) (9.5 x 10^2 CFU/g), spice mix for meatballs (8.0 x 10^2 CFU/g), and pimento (Pimenta officinalis) (7.2 x 10^2 CFU/g). Eight samples (44%) were positive for generic *E. coli*–paprika (*Capsicum annuum*), pimento (*Pimenta dioica*), cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymus vulgaris*), clove (*Eugenia aromaticum*), sumac (*Rhus*) and black pepper (*Piper nigrum*).

Significance: This information could be of interest to food manufacturers, food regulators, and consumers in order to help them to understand the microbial quality of commercial preparations and the potential risk for the presence of bacteria in commercially available products. Future work could focus on enumeration of bacteria from spices using the Most Probable Number (MPN) method and enumeration of spore-forming bacteria, particularly *Bacillus cereus*.

P1-08 Effect of Temperature Abuse on the Organoleptic Quality and Shelf Life of Produce during Simulated Transport Conditions

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Introduction: High temperatures during transport of fresh produce may adversely affect product quality, shelf life and food cost.

Purpose: The purpose of this study was to determine the spoilage effects of temperature abuse on the organoleptic quality and shelf life of produce during simulated transport conditions.

Methods: Iceberg-Romaine mix was subjected to temperature abuse (50°F) for 2, 4, 6, and 8 hours; then held under refrigeration (38°F) throughout shelf life. Produce held at refrigeration temperature throughout shelf life served as a control. Aerobic plate count, *E. coli*/coliform, and yeast/mold analyses (3M Petrifilm Test Kits, 3M Corporation) were run on the product at Days 1, 2, 4, 6, 7, and 8 (end of shelf life) following 3M Petrifilm procedures. Organoleptic observations were performed at each time point.

Results: APC and coliform counts exceeded product specifications by Days 6, 7, respectively, after 4 hours of abuse; yeast counts exceeded product specification by Day 4 after 6 hours of abuse. No *E. coli* was detected and mold growth never surpassed specification. Organoleptic evaluation showed no significant deterioration of product throughout shelf life.

Significance: Data shows that produce subjected to greater than 4 hours at 50°F temperature during simulated transit exceeded specifications for APC, coliforms and yeast before the end of shelf life. If abuse is limited to less than 4 hours at 50°F during transit and produce is used in less than 4 days, no product needs to be rejected.

P1-09 Diffusion-based Time-temperature Indicator for Monitoring the Microbiological Quality of Frozen and Chilled Foods

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Introduction: A time-temperature indicator (TTI) is a simple device attached to products that gives a single visible response indicating cumulative time-temperature history. The TTI response reflects effects of the time-temperature on food quality, allowing the determination of remaining shelf life at any time of distribution or storage period.

Purpose: A prototype diffusion-based TTI system was evaluated to monitor microbiological quality of frozen and chilled foods stored under isothermal or dynamic conditions simulating a frozen or chilled distribution chain.

Methods: A fatty acid ester (FAE) was used for the temperature-dependent diffusion. Diffusion distance of FAE in the TTI system and counts of total aerobic bacteria of chilled angelica juice (unpasteurized) and frozen beef patty (uncooked) were measured under isothermal or dynamic storage conditions for 48 h. Prototype TTI tags were attached to the surface of package of actual food products (chilled angelica juice and frozen beef patty), and tested under normal, mild, and worst scenarios (time-temperature conditions).

Results: FAE diffusion distance of the TTI system corresponded to the storage temperature and time. The counts of total aerobic bacteria were strongly correlated with the TTI diffusion distance. Total aerobic count of 10^6 log CFU/ml in the chilled angelica juice and frozen beef patty were reached after 12.0 h and 18.6 h storage at 25°C, respectively. FAE diffusion of 7.0 mm and 4.6 mm were obtained at the same reaching time to 10^6 log CFU/ml for the angelica juice and beef patty stored at 25°C, respectively. The prototype TTI indicated a positive result (showed a red band mark) when the angelica juice was exposed at 5°C for 30 h and at 25°C for 10 h as an established condition for the worst scenario. Total aerobic bacteria counts of 10^5 ~10^6 log CFU/ml were reached when the TTI started to show positive results in the mild and worst scenarios.

Significance: The diffusion-based TTI developed in our study appeared to have high potential to be used for monitoring microbiological quality of frozen and chilled foods.

P1-10 Control of *Salmonella enterica* and *Staphylococcus aureus* in a Laboratory Medium and a Commercial-type Soup Using Phosvitin, Carvacrol or Combinations

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Introduction: The use of natural antimicrobials from plant, animal or microbial sources has gained significant popularity in the food industry as well as among consumers. Both phosvitin, a phosphoglycoprotein from egg yolk, and carvacrol, an essential oil from oregano or thyme, are natural antimicrobials.

Purpose: This study was conducted to evaluate the antibacterial effect of phosvitin, carvacrol or combinations against *Salmonella enterica* and *Staphylococcus aureus* in brain heart infusion (BHI) broth and onion/mushroom soup.

Methods: Broth with added phosvitin (10, 20, 40, 60, 80 or 100 mg/ml), carvacrol (0.09, 0.12, 0.14, 0.19, 0.38, or 0.75 mg/ml) or combinations was inoculated with a 5-serovar cocktail of *S. enterica* or a 3-strain cocktail of *S. aureus* to give a final concentration of 5.0 log CFU/ml per pathogen. Broth or soup without antimicrobials served as control. Growth of *S. enterica* and *S. aureus* in BHI (35°C, 24 hours) was monitored using a Bioscreen C turbidometer (OD 600nm). Growth of the pathogens in soup (12°C, 8 days) was monitored by surface plating samples on xylose lysine desoxycholate agar (*S. enterica*) and mannitol salt agar (*S. aureus*).

Results: In BHI broth, the minimum inhibitory concentration (MIC) of phosvitin and carvacrol was 80 mg/ml and 0.14 mg/ml, respectively, for *S. enterica*; and >100 mg/ml and 0.12 mg/ml, respectively, for *S. aureus*. In control soup, viable numbers of *S. enterica* increased to 6.95 log CFU/ml at Day 8. Phosvitin (60 mg/ml) or carvacrol (0.19 mg/ml) alone completely suppressed growth of this pathogen. At Day 2, carvacrol (0.38 mg/ml) reduced viable numbers by 3.04 log and survivors remained relatively constant throughout storage (8 days). No growth of *S. aureus* occurred in control or treated soup at 12°C. Phosvitin (60 mg/ml) plus carvacrol (0.38 mg/ml) exhibited the greatest bactericidal effect against both pathogens; at Day 2, viable counts decreased by 3.91 log and 4.43 log, respectively, for *S. enterica* and *S. aureus*. With this combination pathogens were undetected (<10 CFU/ml) from Day 4 through Day 8.

Significance: A combination of phosvitin (60 mg/ml) and carvacrol (0.38 mg/ml) is highly effective for controlling *S. enterica* and *S. aureus* in onion/mushroom soup.

P1-11 The Effectiveness of Plant Compounds/Extracts at Consumer-acceptable Concentrations against *Salmonella* Typhimurium in Ground Pork

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Introduction: A potential method of inhibiting pathogenic bacterial growth in meat is through the introduction of plant-derived antimicrobials. However, while the addition of these antimicrobials may improve the microbiological safety of these products, it is likely that they may simultaneously affect the sensory characteristics of the meat.

Purpose: The purpose of this study was to define the appropriate concentrations of oregano oil (OO), cinnamon oil (CO), olive extract (OE) and apple extract (AE) that are acceptable to consumers when added to ground pork, and to evaluate their antimicrobial activity against *Salmonella* Typhimurium in fresh ground pork.

Methods: To accomplish this, initially a sensory test was conducted to determine the consumer-acceptable concentrations of the four antimicrobials listed above. Experimental groups, which were antimicrobial-treated ground pork, were compared to plain controls. Antimicrobial-treated ground pork was inoculated with *S.* Typhimurium culture (10^7 CFU/ml) and stored at 4°C for up to 7 days. Samples were taken at Days 0, 3, 5, and 7, diluted and plated on appropriate media for enumeration of survivors.

Results: Data were analyzed by one-way ANOVA using Minitab 15.0 software. The acceptable concentrations of OO and CO were 0.5%; OE and AE were 3%. With an addition of 0.5% CO into ground pork, 1.2 and 1.0 log reduction of *S.* Typhimurium were induced at Days 3 and 7. With an addition of 3% OE, 0.9 log reduction of *S.* Typhimuirum was observed at Day 7. Also 1.31 and 3 log reductions were observed in 1.0% CO and 5% OE treated samples. The meat containing OO and AE did not show significant reduction compared to control for the whole test period ($P > 0.05$).

Significance: The results indicated that the antimicrobial activities of the plant compounds against *S.* Typhimurium are limited when added at consumer-acceptable concentrations in ground pork. The antimicrobials were more effective at higher concentrations.

P1-12 Antimicrobial Activity of Plant Extract/Concentrate Rinses against *Salmonella enterica* on Contaminated Organic Leafy Greens

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Introduction: One of the most common bacterial pathogens involved in foodborne outbreaks involving fresh produce in the last decade was *Salmonella enterica*. Because many consumers prefer natural to synthetic compounds, we are evaluating the effectiveness of plant-derived compounds/extracts against *S. enterica* on organic leafy greens.

Purpose: In an effort to discover safe antimicrobials for use on food, the objective was to evaluate the effectiveness of different antimicrobial plant extract/concentrate rinses on four different types of organic leafy greens inoculated with *S. enterica* serovar Newport.

Methods: Leafy green samples tested include organic romaine and iceberg lettuces, and organic adult and baby spinach. Leaf samples were each washed in deionized water, dip inoculated with *S.* Newport (10^6 CFU/mL), and dried for 30 minutes. Apple and olive extract rinses were prepared at 1%, 3%, and 5% concentrations and hibiscus concentrate rinses were prepared at 10%, 20%, and 30% concentrations. Leaves were immersed in the treatment solution for two minutes with gentle agitation, and individually incubated at 4°C. Following incubation, samples were taken at Day 0, 1, and 3, stomached in BPW, then diluted and plated for enumeration of survivors.

Results: The results from this study show that the antimicrobial activity against *S.* Newport was concentration- as well as time-dependent. Olive extract induced the greatest antimicrobial activity, showing reductions of 2-3.1 log CFU/mL for each type of leafy green by Day 3. Reduction in bacterial population on iceberg lettuce was greater than that on the other three leafy greens. Apple extract showed between a 1.0-1.14 log reduction by Day 3 on various leafy greens. The maximum reduction by hydrogen peroxide (3%) was about l log.

Significance: This study demonstrates the potential of natural plant extracts as rinses to inactivate *S.* Newport on organic leafy greens. These extracts can, therefore, find applications in the fresh produce industry as antimicrobial treatments for leafy greens.

P1-13 Evaluation of Cetylpyridinium Chloride to Reduce *Salmonella* and Microbial Counts on Shell Eggs

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Introduction: Shell eggs are the most important vehicle of foodborne illness related to *Salmonella* Enteritidis. Antimicrobial treatments such us ultraviolet light, organic acids, ozone, electrolyzed water and sodium or calcium hypochlorite have been evaluated to reduce microbial populations and improve safety of shell eggs. Cetylpyridinium chloride (CPC) has been used as an antimicrobial agent on beef and poultry carcasses and could be also effective on shell eggs.

Purpose: This study evaluated the effect of cetylpyridinium chloride sprays to reduce *Salmonella* and microbial counts on shell eggs.

Methods: Shell eggs from Hy Line layer hens were collected from a local farm. Eggs were randomly grouped into five-unit composite samples. One set of composite samples was inoculated by immersion in a cocktail of eight *Salmonella* rifampicin-resistant strains to achieve approximately 7 log CFU/egg. The other set was not inoculated and used for enumeration of aerobic plate count (APC), *Enterobacteriaceae* (ENT) and molds/yeasts (M/Y). Treatments applied on the two sets of samples using a spraying cabinet were: 500 mg/L of CPC at 45°C and 55°C, 1000 mg/L of CPC at 45°C and 55°C, 2000 mg/L of a commercial product containing calcium hypochlorite (CH) at 45°C and 55°C, and distilled water at 45°C and 55°C. Immediately after each treatment, samples were rinsed with Dey-Engley neutralizing broth and survivors enumerated. APC, ENT and M/Y counts were performed on Petrifilm plates and *Salmonella* counts on trypticase soy agar with rifampicin (100 µg/ml). Five replicates of each experiment were conducted.

Results: Mean reductions obtained with CPC ranged from 0.9 to 1.8, 1.1 to 1.5, 1.0 to 1.6 and 1.7 to 1.9 log CFU/egg for APC, ENT, M/Y and *Salmonella*, respectively, and were significantly higher ($P < 0.05$) than reductions obtained with CH (0.2 to 0.9 log CFU/egg) and water (0.1 to 0.9 log CFU/egg) treatments for all microbial populations.

Significance: Cetylpiridinium chloride was more effective than a commercial product containing calcium hypochlorite and also than water to reduce *Salmonella* and microbial counts on shell eggs.

P1-14 Antimicrobial Treatments for Reduction of *Salmonella* Contamination in Not Ready-to-Eat, Surface-browned, Frozen, Breaded Chicken Entrees

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Introduction: Not ready-to-eat, frozen, breaded chicken entrees that are surface-browned to induce a desirable golden-brown color, have been identified as sources of salmonellosis outbreaks when consumed without adequate cooking.

Purpose: The aim of this study was to develop antimicrobial interventions for reduction of *Salmonella* contamination in such products.

Methods: Fresh chicken breast meat portions (5×5×5 cm; 2 kg) were inoculated with *Salmonella* (20 ml; 7-strain mixture; 5 log CFU/g) and mixed with distilled water (control) or with each of seven levels of caprylic acid (CAA; 0.03125 to 1.0%), six levels of carvacrol (CAR; 0.0375 to 0.5%) or four levels of ε-polylysine (POL; 0.125 to 0.5%), applied individually or in combinations of two or three, for a total of 25 treatments. Sodium chloride (1.2%) and sodium tripolyphosphate (0.3%) were added to all treatments (5% total moisture enhancement level), and then ground and formed into 9×5×3 cm portions. Samples were glazed with beaten egg whites, rolled in breadcrumbs, browned by oven-baking (208°C, 15 min), packaged in polyethylene bags, and stored at -20°C for 7 days. Samples were analyzed for microbial counts before antimicrobial treatment, and after grinding, browning and frozen storage. Data (two repetitions, three samples/treatment/repetition) were statistically analyzed with independent variables being antimicrobial treatment, sampling point, and their interactions.

Results: Total reductions of inoculated *Salmonella* in control samples ranged from 0.8 to 1.4 log CFU/g after browning and frozen storage. In comparison, single treatments of CAA, CAR or POL reduced counts by 2.9 to >4.5, 3.4 to >4.4 and 1.4 to 2.3 log CFU/g, respectively, depending on concentration. Levels of 0.0625 to 0.25% CAA, 0.075 to 0.3% CAR or 0.5% POL, applied in combinations of two or three, reduced ($P < 0.05$) *Salmonella* counts in stored frozen products by 1.7 to >4.6 log CFU/g. Specifically, combinations of CAA (0.0625 to 0.25%) with CAR (0.075 to 0.3%) reduced *Salmonella* counts by 1.8 to >4.2 log CFU/g. Combinations of 0.0625, 0.125 or 0.25% CAA with 0.5% POL reduced counts by 1.8, 1.7, and 2.6 log CFU/g, respectively, while combinations of 0.075, 0.15 or 0.3% CAR with 0.5% POL reduced counts by 2.0, 3.2, and >4.5 log CFU/g, respectively. Combination of all three antimicrobials reduced ($P < 0.05$) *Salmonella* counts by 2.4 to >4.6 log CFU/g, depending on the concentrations tested.

Significance: These data may be used for the selection of suitable antimicrobials and concentrations to reduce *Salmonella* contamination in not ready-to-eat, surface-browned, frozen, breaded chicken entrees, after evaluation of effects in sensory quality.

P1-15 Reductions of *Salmonella enterica* on Grape Tomatoes during Washing by Thymol and Its Combinations

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Introduction: In recent years, multistate outbreaks of *Salmonella enterica* serovars were traced to tomatoes, and resulted in serious economic loss for the tomato industry and consumer confidence in the safety of tomato produce. Purified compounds derived from essential oils such as thymol had wide inhibitory effects against foodborne pathogens including *Salmonella*.

Purpose: The objective of this study was to develop a formula of thymol or hydrogen peroxide which was combined with organic acid and surfactant to reduce *Salmonella* on grape tomatoes.

Methods: Surface-inoculated grape tomatoes were washed with Thymol (THY) 0.2 mg/ml +SDS 5%+ Acetic acid (AA) 2 mg/ml, 800 ppm H2O2, 200 ppm H2O2 + SDS 4%, and 200 ppm chlorine for 2 min.

Results: THY 0.2 mg/ml +SDS 5%+ AA 2 mg/ml, 800 ppm H2O2, and 200 ppm chlorine achieved around 5.6 log reductions of *Salmonella* on grape tomatoes as compared to real control 4% ethanol. More than 5.0 log reduction in the corresponding used thymol solution was observed in comparison to control. The uses of these antimicrobial agents achieved significant log reductions of *Salmonella* on inoculated grape tomato and decreased dramatically the risk of potential transmission of pathogens from tomatoes to washing solutions. None of these antimicrobial agents changed the color, pH and texture values and affected taste, aroma, visual quality of grape tomatoes.

Significance: Therefore, THY 0.2 mg/ml +SDS 5%+ AA 2 mg/ml, 800 ppm H2O2, has great potential to be an alternative to chlorine-based washing solution for fresh produce.

P1-16 Susceptibility of *Salmonella* spp. Isolated from Soybean Feed Ingredient to Antibiotics

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Introduction: Since the beginning of the 1990s, several reports have been written describing strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans. Attention must be given to the fact that those strains are threatening to become a serious public health problem, since *Salmonella* contamination of animal feed can be disseminated to food producing animals and further down the food chain.

Purpose: The objective of this study was to determine the resistance of twenty two *Salmonella* spp. strains – *S.* Cubana (2), *S.* Albany (2), *S.* Mbandaka (5), *S.* Alachua (3), *S.* Morehead (1), *S.* Livingstone (2), *S.* Anatum (2), *S.* Schwarzengrund (2), *S.* Rissen (2) and *S.* Tennessee (1) – isolated from soybean feed ingredient to various antimicrobial agents.

Methods: The *Salmonella* spp. strains were tested for antimicrobial resistance by the disk diffusion method on Mueller-Hinton agar, according to the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The following antimicrobial agents were tested: ampicillin 10 µg, ceftriaxone 30 µg, ceftazidime 30 µg, tetracycline 30 µg, gentamicin 10 µg, streptomycin 10 µg, chloramphenicol 30 µg, trimethoprim 5 µg, nalidixic acid 30 µg, ciprofloxacin 5 µg and iminepem 10 µg.

Results: Antimicrobial resistance analysis indicated that all *Salmonella* spp. studied were susceptible to the antibiotics tested.

Significance: Infection caused by these isolates can be appropriately addressed with the dosage of an antimicrobial agent recommended for this type of infection and pathogen, unless otherwise indicated.

P1-17 Minimum Inhibitory and Bactericidal Concentrations of Phosphoric Acid, Acidified Calcium Sulfate and Stabilized Hydrogen Peroxide-based Oxidizer against *Salmonella* Montevideo and *Staphylococcus aureus*

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Introduction: Use of antimicrobials in the food industry continues to be a critical element of integrated food safety strategies.

Purpose: This study was done to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of three FDA approved antimicrobials [phosphoric acid, PA; acidified calcium sulfate, ACS; and Boxyl® powder (stabilized hydrogen peroxide-based oxidizer), BP] against *Salmonella* Montevideo and *Staphylococcus aureus*.

Methods: Tryptic soy broth (TSB) containing 0 to 10% PA, or combinations of ACS (0 to 0.5%) and BP (0 to 600 ppm), was used as the test medium. TSB tubes (9 mL) containing various concentrations of antimicrobials were inoculated with 1 mL of a 24-h old target culture and incubated at 37°C for *Salmonella* Montevideo and 35°C for *Staphylococcus aureus*. TSB tubes were observed after 24 h for visible turbidity, indicating growth; tubes with no growth were transferred to D/E Neutralizing broth and incubated for an additional 24 h. Differences ($P \leq 0.05$) in pH mean values were determined using a 1-way ANOVA for PA and a 2-way ANOVA for ACS and BP.

Results: The MIC of PA against *Salmonella* Montevideo and *Staphylococcus aureus* was 3.0% (pH 3.45) and the MBC was 3.5% (pH 3.19). The MIC and MBC of BP alone against *Salmonella* Montevideo were 350 ppm (pH 7.41) and 400 ppm (pH 7.43), respectively, and against *Staphylococcus aureus* the MIC and MBC were 200 ppm (pH 7.37) and 300 ppm (pH 7.39), respectively. ACS alone was able to inhibit the growth of *Salmonella* Montevideo at 0.5% (pH 3.90) and *Staphylococcus aureus* at 0.45% (pH 4.25); but did not have a bactericidal effect at the concentrations evaluated. For *Salmonella* Montevideo, the combination of 0.05% ACS/300 ppm BP inhibited growth and 0.05% ACS/550 ppm BP was bactericidal; whereas for *Staphylococcus aureus*, the MIC and MBC were 0.05% ACS/200 ppm BP and 0.05% ACS/500ppm BP, respectively.

Significance: Using PA, ACS, and/or BP can restrict the growth of *Salmonella* Montevideo and *Staphylococcus aureus*; however, the above-determined MIC and MBC should be validated using various food matrices. The effect of these antimicrobials on the sensory attributes should also be studied.

P1-18 Antimicrobial Resistance of *Salmonella* Isolates Obtained from Beef Carcasses, Beef Chunks and Ground Beef in Mexico

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Introduction: Human salmonellosis is frequently related to consumption of foods of animal origin including beef products. The presence of multidrug-resistant *Salmonella* isolates on foods including beef products may reduce therapeutic options if those are involved in foodborne infections. Limited studies about antimicrobial resistance of *Salmonella* isolates at different stages of the beef chain have been reported. Antimicrobial resistance of *Salmonella* may be different among isolates from beef carcasses and beef products at retail.

Purpose: To compare the antimicrobial resistance of *Salmonella* isolates recovered from beef carcasses at abattoirs and from ground beef and beef chunks obtained from butcher shops in Jalisco State, Mexico.

Methods: Antimicrobial susceptibility of 419 *Salmonella* isolates obtained from beef carcasses (n=106), ground beef (n=193) and beef chunks (n=120) was investigated using the disk diffusion method. Antimicrobials tested were ampicillin, cephalothin, ciprofloxacin, ceftriaxone, chloranphenicol, gentamicin, kanamycin, nalidixic acid, tetracycline, streptomycin and trimethoprim-sulfamethoxazole.

Results: Frequency of *Salmonella* isolates resistant to at least one antimicrobial was 59.4, 65.8 and 72.5% for beef carcasses, ground beef and beef chunks, respectively. Resistance to tetracycline was the most commonly observed (57.5% of total isolates) followed by resistance to streptomycin (43.2%) and nalidixic acid (44.1%). Twelve, 25 and 31 different resistance profiles were identified in isolates from beef carcasses, ground beef and beef chunks, respectively. Multiresistance to at least 3 antimicrobials was observed in 45 of 63 resistant isolates from beef carcasses, in 59 of 87 resistant isolates from beef chunks and 72 of 127 resistant isolates from ground beef. Multiresistance was most commonly observed in *Salmonella* Typhimurium isolates.

Significance: A high frequency of antimicrobial resistance and multiresistance was observed among *Salmonella* isolates obtained from raw beef. Frequency of resistant isolates as well as diversity of resistance profiles increases at further stages of the beef chain.

P1-19 Control of *Listeria monocytogenes* in Sliced Pork Bologna by Bacteriocins Produced by a New *Lactobacillus sakei* Strain

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Introduction: Sliced delicatessen meat products are ready-to-eat products that may contain *Listeria monocytogenes* due to post processing contamination. Bacteriocins produced by lactic acid bacteria have a potential technological application in inhibiting the growth of this psychrotrophic pathogen in these kind of products during cold storage.

Purpose: This study aimed to isolate a bacteriocin-producing strain of lactic acid bacteria from pork bologna and verify the effectiveness of the partially purified bacteriocin on the control of *L. monocytogenes* strains in experimentally inoculated sliced pork bologna, during storage under refrigeration.

Methods: Bacteriocin-producing strains of lactic acid bacteria were isolated from sliced pork bologna using the triple-agar method. One strain, identified as *Lactobacillus sakei* by 16S gene sequencing by PCR, was selected and used for preparation of an acid extract containing a partially purified bacteriocin, obtained from a 24-h culture in MRS broth. The activity of the partially purified bacteriocin was 204.800 IU/mL. Sliced bologna samples were experimentally contaminated with a pool of ten *L. monocytogenes* strains (10^3 CFU/mL), previously isolated from meat products. The bacteriocin extract was added to the surface of individual slices of bologna (250 µl per slice), that were then vacuum packed and kept at 7°C up to 40 days. Controls with no added bacteriocin were also prepared. Counts of *L. monocytogenes* in the slices were performed every 5 days.

Results: L. monocytogenes did not grow in the bologna slices, but in those containing the bacteriocin extract the counts were reduced to 2 log CFU/g after 24 h and remained 3 log CFU/g in the control samples. This one log difference was maintained up to 40 days of storage under refrigeration.

Significance: The partially purified bacteriocin extract produced by the new *L. sakei* strain showed potential use for reducing the counts of *L. monocytogenes* in sliced pork bologna.

P1-20 Inhibition of *Listeria monocytogenes* on a Processed Meat Product Manufactured with Combinations of Bacteriocin Biopreservative and Chemical Preservatives

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Introduction: Biopreservative cultures and the metabolites they produce are effective hurdles to the growth of *Listeria monocytogenes* in ready-to-eat meats. The use of multiple hurdles may enhance the antimicrobial efficacy of biopreservatives.

Purpose: The objective of the study is to evaluate the anti-listerial effect of adding different combinations of chemical preservatives (acetates, lactates and organic acids) with a bacteriocin-based biopreservative, into wieners.

Methods: The biopreservative was produced by a strain of bacteriocinogenic *Carnobacterium maltaromaticum* CB1. All inhibitory agents were added to the formulation prior to processing. The biopreservative Micocin® (produced by *C. maltaromaticum* CB1) was added at 0.1 and 0.25% and the concentration of chemicals were evaluated at recommended use levels. After processing, the wieners were surface inoculated to give approximately 1 x 10^3 of a cocktail of *L. monocytogenes* (including serotypes 1/2a, 1/2b, 3a and 4b) per gram of product. The inoculated wieners were vacuum-packaged, stored at 4°C and *Listeria* were enumerated on PALCAM agar during 14 weeks of storage.

Results: In the presence of the chemicals and biopreservative combinations *Listeria* did not increase in numbers for the entire testing period (total of 14 weeks) and on average reduced from the level of inoculation by approximately 10^1 CFU/g, which is below the level of detection using standard plating technique. In contrast, the *Listeria* control (no antimicrobials) reached 10^8 CFU/g within 14 weeks of storage, making an approximate 7-log differential between the control and products that contained different combinations of bio- and chemical preservatives.

Significance: The addition of a combination of the bacteriocin-based biopreservative and chemical antimicrobials to wieners prior to processing could allow meat processors to easily and effectively reduce the potential for *Listeria monocytogenes* detection and outgrowth in their processed meat products.

P1-21 Antilisterial Activity of Lactic Acid Bacteria Isolated from Naturally Fermented Sausages

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Introduction: During sausage fermentation and drying processes, *L. monocytogenes* numbers tend to decrease substantially because of the inhibitory environment created by a set of hurdles (low pH, low a_w and high salt concentration). However, *L. monocytogenes* demonstrates high tolerance to environmental stress and may thus be particularly likely to survive the steps involved in the manufacture of fermented and dried meat products. A potential means of inhibiting growth of *L. monocytogenes* in dry fermented sausages is the use of bacteriocin-producing starter cultures.

Purpose: The aim of this present study was to test the antilisterial activity and the characterization of antibacterial compounds of 279 lactic acid bacteria (LAB) isolated from naturally fermented sausages produced in the southern region of Brazil.

Methods: Four strains of *L. monocytogenes* were used as target bacteria for the inhibitory effects of LAB. Three strains were isolated from naturally fermented sausages and an ATCC 7644 strain. For detection of antagonistic activity, an agar spot test and a well diffusion assay were used. The agar spot test was performed according to Lewus et al. (1991). Isolates exhibiting antagonistic activities against *L. monocytogenes* were then checked by a well diffusion assay and the sensitivity to heat and proteolytic enzymes was tested as described by Ammor et al. (2006).

Results: A total of 30 of 279 (10.75 %) isolates of LAB were found to produce inhibition zones against strains of *L. monocytogenes* on agar spot test. The culture supernatants of only four (1.43%) isolates produced inhibition zones on agar. The activity of the bacterial compound produced by the four isolates of LAB was destroyed by protease treatment but was resistant to heat. These results demonstrate that the antimicrobial compound produced by LABs is a heat-stable peptide or protein.

Significance: The low percentage of LAB isolates that showed antilisterial activity is important in terms of public health because fermented sausages are ready-to-eat products and may serve as vehicles for *L. monocytogenes*. The production of bacteriocins especially directed to foodborne pathogens is an important characteristic when selecting LAB starter cultures for meat products.

P1-22 Effects of Lactate, Diacetate and Nisin on the Cold Growth of *Listeria monocytogenes* in a Broth System

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Introduction: Organic acids, bacteriocins and their combinations are used in ready-to-eat seafood products to control the growth of *Listeria monocytogenes* at refrigeration temperatures. There is a need to quantify their effects on *Listeria monocytogenes'* growth and identify potential synergies.

Purpose: The purpose of this study was to test the efficacy of different growth inhibitors and their binary combinations on the cold growth of a diverse set of *L. monocytogenes* strains in a broth system with pH 6.1 and 4.65% water phase salt, similar to cold-smoked salmon.

Methods: Twenty strains of *L. monocytogenes* were grown at 7°C in brain heart infusion broth supplemented with potassium lactate (2% water phase concentration), sodium diacetate (0.14% water phase), nisin (50 ppm), the combination of two inhibitors at the same levels, or without inhibitors as the control.

Results: Addition of nisin significantly lowered the initial cell density (N_0) from 6.05 ± 0.45 to 4.48 ± 1.10 $\log_{10}$(CFU/ml), while lactate lowered the maximum cell density (N_{max}) from 8.90 ± 0.16 to 8.32 ± 0.16 $\log_{10}$(CFU/ml). Nisin+diacetate significantly extended the lag phase (λ) from 2.64 ± 0.54 to 5.22 ± 2.76 days. Nisin+lactate synergistically extended λ from 2.64 ± 0.54 to 10.00 ± 5.09 days, which was similar to the effect of the combination of lactate+diacetate (12.17 ± 4.53 days), and lowered N_{max} from 8.90 ± 0.16 to 7.97 ± 0.45 $\log_{10}$(CFU/ml).

Significance: While nisin alone does not significantly affect λ, N_{max}, or maximum growth rate, it does decrease N_0. Further, the combination treatment of nisin with an organic acid significantly extends λ and lowers the N_{max}, and nisin synergistically enhances the effect of potassium lactate on extending λ of *Listeria monocytogenes* at refrigeration temperature. These data suggest that nisin in combination with an organic acid, such as lactate, would both reduce initial levels of *Listeria monocytogenes* and delay their re-growth on ready-to-eat foods.

P1-23 Effect of Liquid Smoke as an Ingredient on *Listeria monocytogenes* and Quality Attributes of Frankfurters

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Introduction: Changes in market trends indicate an increased interest in 'natural antimicrobials' to augment safety of ready-to-eat meat and poultry against *Listeria monocytogenes*. Liquid smoke, an all natural condensate of smoke components, applied as a post-process lethality treatment exhibits anti-listerial properties. Topical application by either spray or immersion requires additional infrastructure, drain time, and drip handling, hence delaying packaging. These impediments can be overcome by incorporating liquid smoke as an ingredient but studies on its antimicrobial effect and quality attributes are lacking.

Purpose: The aim of this study was to validate and optimize incorporation of liquid smoke as an ingredient in frankfurters and determine its antimicrobial effect against *L. monocytogenes* in addition to its impact on microbial shelf life without affecting quality attributes.

Methods: Chicken frankfurters were fabricated with pork fat, frankfurter seasoning, and 0, 2.5, 5 and 10% liquid smoke. No other antimicrobials were added to the formulations. Cooked casing-stripped frankfurters were spray inoculated with either high (6 $\log_{10}$ CFU/ml) or low (3.5 $\log_{10}$ CFU/ml) levels of *L. monocytogenes* serotype 4b. Following 30 min of attachment time, inoculated frankfurters (4 per package) were vacuum packaged and stored at 4°C for up to 21 days. Samples were taken every week from day of inoculation (Day 0) for 21 days. For microbiological sampling (3 bags/treatment/sampling), the entire content of each bag was rinsed with 25 ml of peptone water for 2 min, and 0.1 ml was then spread plated on differential media for estimation of *L. monocytogenes*, aerobic plate counts, yeast and molds, lactic acid bacteria, total coliforms, and *Escherichia coli*. Non-inoculated frankfurters from each treatment were used for sensory analysis, texture profile analysis, and color (L* a* b*) measurement.

Results: Incorporating smoke extract at 2.5, 5, and 10 % significantly reduced ($P < 0.05$) populations of *L. monocytogenes* as compared to the controls throughout the storage period irrespective of the inoculation levels. Furthermore, incorporation of smoke extract significantly decreased L* ($P < 0.05$), increased b* ($P < 0.05$) and hardness ($P < 0.05$).

Significance: Liquid smoke can be effectively incorporated as an 'all natural' listeriostatic ingredient in the manufacture of frankfurters without potentially affecting quality attributes such as color and texture.

P1-24 Inactivation of *Listeria monocytogenes* on Ham and Bologna Using Apple-, Carrot-, and Hibiscus-based Edible Films Containing Carvacrol and Cinnamaldehyde

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Introduction: Edible films are used as wrapping material on food products to reduce surface contamination. Natural products derived from plants have gained popularity in recent years as antimicrobials in food processing to render a safe food product for the consumer. The incorporation of antimicrobials into edible films could serve as an additional barrier against pathogenic and spoilage microorganisms that contaminate food surfaces.

Purpose: The objective of this study was to investigate the antimicrobial effects of carvacrol and cinnamaldehyde incorporated into apple-, carrot- and hibiscus-based edible films against *Listeria monocytogenes* on contaminated ham and bologna.

Methods: Ham or bologna samples were inoculated on both sides with 0.1 ml of 10^6 CFU/ml *L. monocytogenes* culture. Samples were dried under a biohood for 30 min and then surface wrapped with apple, carrot or hibiscus films containing 0.5, 1.5 and 3% carvacrol or cinnamaldehyde. The samples were stored at 4°C for 7 days. Samples were taken for plating and enumeration of survivors on Days 0, 3 and 7.

Results: Carvacrol films showed stronger activities than cinnamaldehyde films. Compared to controls, films with 3% carvacrol showed 1–3, 2–3 and 2–5 log reductions on ham and bologna at Day 0, 3, and 7, respectively. Reductions with 1.5% carvacrol were 0.5–1, 1–1.5 and 1–2 logs at Day 0, 3 and 7, respectively. At Day 7, films with 3% cinnamaldehyde reduced *L. monocytogenes* populations by 0.5–1.5 and 0.5–1.0 logs on ham and bologna, respectively. Inactivation by apple films was greater than that by carrot or hibiscus films. Apple films containing 3% carvacrol reduced the listerial population on ham by 3 logs on Day 0, which was 1–2 logs greater than that by carrot and hibiscus films. Films were more effective on ham than on bologna.

Significance: The food industry and consumers could use these films to control surface contamination by pathogenic microorganisms.

P1-25 Effect of Oleanolic Acid on Inhibition of *Listeria monocytogenes* Growth under Food-related Conditions

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Introduction: Recently, consumers have preferred use of antimicrobials of plant origin to synthesized chemical antimicrobials to inhibit *Listeria monocytogenes* growth in ready-to-eat meat and poultry.

Purpose: The objective of this study was to evaluate the antimicrobial activity of plant-originated oleanolic acid on inhibition of *L. monocytogenes* growth under food-related stresses such as NaCl and low pH.

Methods: L. monocytogenes ATCC15313 (6 log CFU/ml) was inoculated in microplate wells containing brain heart infusion (BHI) broth formulated with combinations [oleanolic acid (0, 0.25, 0.5, 1.0, 1.5, 2.0, and 4.0 μg/ml)x pH (5 and 7) x NaCl (0, 3, and 6%)]. The microplates were incubated under accelerated storage condition at 37°C for 48 h, and optical density (OD) of the samples was measured at 0, 6, 12, 24, and 48 h at 600 nm. All least squares mean comparisons among the interactions of treatments were performed with the pairwise t-test at alpha = 0.05.

Results: After lag phase duration was observed at early stage of incubation, the OD values of *L. monocytogenes* significantly increased ($P < 0.05$) in BHI broth supplemented with 0 and 3% of NaCl at pH 5 and 7 during storage. However, *L. monocytogenes* experienced 6% NaCl had no growth and gradually increased growth only at less than 0.5 μg/ml of oleanolic acid for pH 5 and 7, respectively. Moreover, *L. monocytogenes* generally had lower OD values as the concentrations of oleanolic acid increased. As expected, the OD values of *L. monocytogenes* were generally higher ($P < 0.05$) in pH 7 than in pH 5.

Significance: These results suggest that oleanolic acid could be used as a phytochemical in inhibiting *L. monocytogenes* growth in various foods.

P1-26 Anti-listerial Effects of Sodium Metasilicate in Pure *Listeria monocytogenes* Culture Suspensions and on Ready-to-Eat Turkey Ham

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Introduction: Listeria monocytogenes is a foodborne pathogen of high public health significance and frequently encountered in ready-to-eat foods. Sodium metasilicate (SMS) is a USDA-approved antimicrobial for use in ready-to-eat meat and poultry products.

Purpose: The purpose of this study was to determine effectiveness of SMS for inactivating *L. monocytogenes* in pure culture suspensions and on ready-to-eat turkey ham.

Methods: L. monocytogenes Scott A strain was exposed to 0 (positive control), 1, 2, 3, 4, 5 and 6% SMS solutions for 1, 10 and 30 min at room temperature. In a second experiment, turkey ham samples were inoculated with *L. monocytogenes* (Scott A) and treated with SMS to yield final concentrations of 300 and 600 ppm, vacuum packaged, stored at 4°C and analyzed after 0, 7, 14, 21 and 28 days for presence of *L. monocytogenes* and pH. Negative and positive controls were also included. Both experiments were replicated three times and all the samples were analyzed in duplicate.

Results: In Experiment 1, no significant reduction in *L. monocytogenes* ($P > 0.05$) was observed with 1 min exposure to 1.0 and 2.0% SMS. Concentrations of 1.0, 2.0 and 3.0% SMS reduced *L. monocytogenes* by more than 5 log after 30 min. Exposure for 1 min to 4.0 and 5.0% SMS reduced *L. monocytogenes* by more than 5 log. *L. monocytogenes* was not detected after 1 min exposure to 6.0% SMS. In Experiment 2, 600 ppm SMS was not effective in reducing *L. monocytogenes* populations ($P > 0.05$) in turkey ham, and pH values were similar ($P > 0.05$) for all treatments.

Significance: L. monocytogenes was susceptible to SMS in pure culture. Susceptibility of *L. monocytogenes* to SMS was reduced significantly when *L. monocytogenes* was inoculated onto ready-to-eat turkey ham, which suggested that higher concentrations of SMS may be needed to control growth of *L. monocytogenes* on ready-to-eat poultry products.

P1-27 The Biosynthesis of Paenibacillin and Its Use against *Listeria monocytogenes* in Meat Products

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Introduction: Paenibacillus polymyxa OSY-DF produces two antimicrobials, polymyxin E1 and paenibacillin, a novel lantibiotic. The primary structure of paenibacillin has been determined by NMR and MS/MS analyses previously by our group, but its genetic code has not been revealed. Additionally, usefulness of paenibacillin in food applications has not been investigated.

Purpose: The objectives of this study were to (i) identify the gene cluster encoding paenibacillin, and (ii) test the feasibility of using the lantibiotic to control *Listeria monocytogenes* in meat.

Methods: While searching for paenibacillin gene cluster, a putative lantibiotic dehydratase (*paenB*) gene was amplified by PCR using primers based on the conserved domain of known genes. To obtain the DNA sequence flanking *paenB*, the upstream and downstream, DNA was amplified by PCR using a gene-specific primer and an universal primer from GenomeWalker Universal Kits (Clontech). In an eight-kb fragment, putative lantibiotic dehydratase (*paenB*), lantibiotic cyclase (*paenC*) and ABC transporter (*paenT*) genes were identified. However, the structural gene encoding paenibacillin precursor has not been found within this fragment. To correlate *paenB*, *paenC* and *paenT* with post-translational modification and lantibiotics transportation, targeted gene inactivation will be performed by homologous recombination using plasmid pMutin4 (BGSC).

Results: To address Objective 2, crude extract (CE) of paenibacillin was prepared by ion-exchange chromatography and solid-phase extraction using Sep-Pak C18 cartridges. Adding 3.3% (v/w) CE to Vienna sausage inhibited the growth of *L. monocytogenes* when the product was incubated for 24 h at 25°C. Under similar conditions, the population of *Listeria* increased from 5.0 logs to 8.7 logs in the untreated control. Similarly, paenibacillin inhibited the growth of *L. monocytogenes* in irradiated ground beef while being held at 4°C for 7 days.

Significance: In conclusion, the novel lantibiotic (paenibacillin) appears to be useful in food applications to control *L. monocytogenes*. The knowledge about its biosynthesis may help to modify the native paenibacillin structure for higher activity and broader inhibitory spectrum.

P1-28 Genotypic and Phenotypic Properties of *Listeria monocytogenes* Strains Isolated from Ready-to-Eat Foods and Food-processing Environments in British Columbia, Canada

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Introduction: Listeria monocytogenes (Lm), a microorganism capable of causing severe disease in humans, is a frequent issue in food premises and ready-to-eat (RTE) products in Canada. In general, *Lm* is considered to be sensitive to most antimicrobials. However, there are limited data detailing antimicrobial resistance in *Lm* originating from British Columbia (BC).

Purpose: The intent of this study was to examine genotypic and phenotypic properties, including antibiogram typing, of *Lm* strains recovered from the BC food supply.

Methods: In total, 54 isolates were characterized: 29 from food processing environments, 19 from RTE foods, and six from raw foods. All isolates were serotyped by slide agglutination and screened by PCR for the presence of LGI1 genomic island. Antimicrobial resistance was determined using disc diffusion assay according to CLSI guidelines. In total, a panel of 18 antimicrobials comprising 11 classes of antibiotics was used.

Results: The majority (39%) of *Lm* isolates belonged to serotype 4b followed by 1/2a (37%), 1/2c (15%), 3a (7%) and 1/2b (2%). All isolates were sensitive to amikacin, ampicillin, chloramphenicol, cotrimoxazole, erythromycin, gentamicin, kanamycin, imipenem, trimethoprim and vancomycin, while all were resistant to nalidixic acid. Resistance to cefoxitin (98%), ciprofloxacin (7%), clindamycin (33%) and tetracycline (6%) was also observed, as was reduced susceptibility to ciprofloxacin (67%), clindamycin (65%), linezolid (6%), rifampin (2%) and streptomycin (6%). LGI1 was not detected in any of isolates.

Significance: Serotypes 4b and 1/2a, responsible for the majority of listeriosis outbreaks in Canada, are commonly found in foods and food-processing environments in BC. These isolates do not appear to be resistant to antibiotics used in the treatment of listeriosis; however, reduced susceptibility in a significant proportion of strains to clinically relevant antibiotics highlights the need for continuous antimicrobial surveillance of *Lm* strains recovered from the food chain.

P1-29 Fate of *Listeria monocytogenes* on Ham and Turkey Breast Formulated with Lactate-diacetate and Inoculated with the Pathogen at Different Stages of Their Shelf Life

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Introduction: Sodium/potassium lactate and sodium diacetate are included in ready-to-eat (RTE) meat and poultry product formulations, by the U.S. meat industry, to control the growth of *Listeria monocytogenes* contamination potentially introduced after processing. Although the anti-listerial effects of lactate-diacetate are well-documented, research studies have mainly focused on contamination scenarios of freshly-prepared RTE meat products and subsequent storage. However, it is possible that pathogen contamination could occur at different stages of the shelf life of products. As such, the long-term anti-listerial effects of lactate-diacetate need to be elucidated.

Purpose: This study evaluated survival/growth of *L. monocytogenes* on ham and turkey breast products formulated with lactate-diacetate and inoculated with the pathogen at different stages of their shelf life.

Methods: Cured ham and turkey breast products formulated with potassium lactate and sodium diacetate (2.07 and 0.15%, respectively for ham, and 2.24 and 0.16%, respectively for turkey breast) were prepared by a commercial manufacturer, and were stored unsliced in their original processing bags at 1.7°C for up to 180 days. On Days 5, 90, 120, and 180 of storage, products were sliced (10.2×7.6 cm, 1–2 mm thick) and inoculated (1–2 log CFU/cm^2) with a 10-strain mixture of *L. monocytogenes*. Inoculated slices were vacuum-packaged (seven slices/sample), stored at 4°C (13 weeks), and periodically analyzed (two repetitions, three samples/product/repetition) for total, *L. monocytogenes*, lactic acid

bacteria, and yeast and mold counts. Data were analyzed as a randomized complete block design, with independent variables including product age before slicing and inoculation, storage time of sliced inoculated products, and their interaction.

Results: Overall, the anti-listerial activity of lactate-diacetate during 13 weeks of vacuum-packaged storage at 4°C of sliced, inoculated ham and turkey breast, was not ($P \geq 0.05$) affected by the age (up to 180 days post-production) of the products prior to slicing and contamination with the pathogen. Initial levels of inoculated *L. monocytogenes* on ham and turkey breast were 1.4 to 1.5 log CFU/cm^2, and after 13 weeks at 4°C, pathogen counts were 1.5 to 2.3 (ham) and 2.3 to 2.5 (turkey breast) log CFU/cm^2, regardless of product age before slicing and inoculation.

Significance: These data indicate that the anti-listerial activity of lactate-diacetate was not affected by the age of the products before contamination with the pathogen. These findings will be useful to the meat industry and risk assessors in their efforts to control *L. monocytogenes* in RTE meat products.

P1-30 Antibacterial Activity of Dried Lily Flowers against *Escherichia coli* O157:H7

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Introduction: Finding natural antimicrobial ingredients to enhance the safety of food products is of great interest to the food industry because of the high cost of foodborne infections. Dried lily flowers (*Lilium candidum*) have a fruity, floral scent and have been used in the traditional Chinese dishes such as golden needles chicken and sweet and sour soup.

Purpose: The objective of this study was to determine the antibacterial activity of dried lily flowers against *E. coli* O157:H7 in culture medium BHI.

Methods: Escherichia coli O157:H7 culture with approximate populations of 3 logs CFU/ml were individually inoculated into BHI broth samples containing different concentrations of xoconostle extract. Samples were individually incubated at 37°C for 8 h. During the incubation period, bacterial growth was determined (turbidity via optical density at 610 nm) at 2 h intervals. At the end of the incubation period, the BHI broth was also diluted in sterile 0.05% peptone water and then surface plated onto BHI agar. Agar well diffusion assay was used to determine the minimum inhibitory volume (MIV) (the lowest volume that inhibit growth) and the minimum lethal inhibition volume (MLV) (the lowest volume that shows significant growth inhibition within three days of incubation).

Results: Results showed that bacterial population in control reached 8–9 log CFU/ml, while the addition of 10% dried lily flowers extract caused the bacterial population to remain within 3–4 log CFU/ml. Dried lily flowers extract has a strong antibacterial activity against *E. coli* O157:H7 where the zone of inhibition was 10.2 ± 0.02 mm.

Significance: These results indicated that dried lily flowers have potential antibacterial effects against the growth *Escherichia coli* O157:H7. However, additional research is needed to explore its potential antimicrobial effects in the food matrix.

P1-31 Simultaneous Inactivation of *Escherichia coli* O157:H7 and Reduction of Potentially Carcinogenic Heterocyclic Amines in Grilled Hamburger Patties by Plant Compounds

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Introduction: Meat products need to be sufficiently heated to inactivate foodborne pathogens. However, high-temperature heat treatment of meats could cause a serious health concern; formation of potentially carcinogenic heterocyclic amines (HCAs). There is a need to develop methods to cook the meats such that bacteria are inactivated at lower temperatures, while HCAs are concurrently reduced. Natural plant compounds having antimicrobial and antioxidative activity may serve such purpose.

Purpose: Evaluate the ability of plant compounds to simultaneously reduce *E. coli* O157:H7 and heterocyclic amines in heated hamburger patties.

Methods: Ground beef with added plant compounds was inoculated with *E. coli* O157:H7 (10^7 CFU/g). The beef was made into patties and two approaches were used for heat treatment, a) cooked to reach the required internal temperatures (70–90°C) at the geometric center and b) patties were cooked for 5 min. Samples were taken for microbiological analysis and for determining the HCA content after cooling. Samples were plated to enumerate surviving bacteria. HCA compounds were extracted from the patties by solid phase extraction and HCA content determined by Mass Spectrometry.

Results: Compared to non-heated controls, HCA levels increased by 2.8–6.2%, 5.4–60.6% and 37.8–40.4% in patties cooked at 70, 80 and 90°C, respectively. When heating the patties for 5 minutes, inhibition of HCAs by apple skin, olive, green tea and grape seed extracts (5%) ranged from 17.8–85.9%. *E. coli* O157:H7 was reduced to below detection with the addition of olive and apple skin extracts. Green tea and grape seed extracts reduced the *E. coli* O157:H7 population by 1.62 and 2.17 logs, respectively. Addition of 1% carvacrol, the main ingredient of oregano oil, to hamburger patties resulted in simultaneous reduction of *E. coli* O157:H7 and HCAs. *E. coli* O157:H7 was reduced by 2.5–5.0 logs at various temperatures, while the greatest reductions in HCA levels were seen at 70°C; MeIQ, MeIQx and PhIP were reduced by 58%, 72%, and 78%, respectively.

Significance: These results imply that it may be possible to reduce the need to consume well-done meat and suggest that the use of lower temperatures to cook ground beef with plant compounds has the potential to improve microbial food safety and prevent cancer.

P1-32 Evaluation of Antimicrobials for Simultaneous Inhibition of *Escherichia coli* and *Pseudomonas* spp. in Injection Brine

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Introduction: In recent years, brine injected meat has become more prevalent on supermarket shelves. This poses new risks to consumers and processors, as spoilage and potentially pathogenic bacteria may survive in injection brine and increase the risk of contamination during processing. They may also survive the cooking process.

Purpose: The objective was to study the efficacy of commercially-approved antimicrobials for the inhibition of *Pseudomonas* spp. and *E. coli* in commercial brine solution.

Methods: Multiple strains of *Pseudomonas* and heat-resistant *E. coli* and *E. coli* O157:H7 were grown in tryptic soy or Luria-Bertani broth at 25°C (*Pseudomonas* spp.) or 35°C (*E. coli*) for 24 h. Cells (log 6 CFU/well) were added to wells containing antimicrobials (extracts of green tea, rosemary and cranberry, and Charsol® RA07015: Micocin X™ and PuraQ™ Verdad™ NV55 with or without 0.5mM EDTA) diluted in brine (0.8% NaCl; 0.5% sodium tripolyphosphate, final concentration) and plates were incubated at 25°C or 35°C for 24 h, respectively. Minimum bactericidal concentrations (MBC) were determined. Experiments were replicated 3 times.

Results: Green tea extract and Charsol® inhibited the growth of *E. coli* and no survival was detected at 1 or 0.63%, respectively. The fractional bactericidal concentration index for a combination of these two compounds was 4, which indicates no interaction or antagonism between the antimicrobials against a cocktail of four strains of *E. coli*. The response of *Pseudomonas* spp. to antimicrobials was variable; however, green

tea extract and Charsol® were most effective on their own. Both Micocin X™ and PuraQ™ with 0.5mM EDTA had MBC ranging from 0.03% to 0.00025% and 0.16% to 0.00025%, respectively.

Significance: Antimicrobials can potentially be used in brine solutions to eliminate pathogenic and spoilage bacteria, which limits the potential for continuous contamination of meat during processing.

P1-33 New Surfactant and Organic Acid Combination Treatments for Inactivation of *Escherichia coli* O157:H7 Strain 87-23

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Introduction: Many of the foodborne pathogen intervention methods involve the use of chemical antimicrobial agents such as Ca(OCl)2. Although the chemical methods are popular because of their convenience and economy, they have shown limited effectiveness in food surface sanitation applications and sometimes an adverse effect on consumers. There is a need to develop new sanitizing agents with an enhanced antimicrobial potency and minimal impact on the environment and consumers.

Purpose: The objective of this study was to explore the use of DC-11 in combination with organic acids as a new sanitizing agent for microbial inactivation.

Methods: Escherichia coli O157:H7 cells suspended in peptone water were treated with 0.01~0.05% DC-11, 0.1%~0.5% organic acid, or combinations of DC-11 and acetic acid. Six organic acids were tested to select the one with the best synergistic effect with DC-11. The samples treated for a specific time period were collected and spread on tryptic soy agar, followed by an overnight incubation at 37°C.

Results: An increase in the concentration of DC-11 or organic acid enhanced the bacterial killing. The DC-11 alone was more effective than the organic acids alone. The *E. coli* cells were reduced to undetectable levels (> 6 log reduction) by a combined treatment with > 0.03% DC-11 and > 0.3% acetic acid for less than 1 min. All the organic acid and 0.05% DC-11 combinations reduced the *E. coli* populations to undetectable levels. With the combinations of DC-11 and malic acid or formic acid, the cells were eliminated in a few seconds.

Significance: The combined treatments of the new sanitizing compound (DC-11) and an organic acid exhibited a synergistic effect on the inactivation of *E. coli* O157:H7. The new formula may be used as an effective sanitizer for the inactivation of human pathogens on surfaces of fresh produce and meat products.

P1-34 Inactivation of *Escherichia coli* O157:H7 Strain 87-23 on Fresh Produce, Beef and Chicken with a New Sanitizer

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Introduction: Processing facilities of fresh produce, meat, and poultry are constantly seeking appropriate sanitation methods to reduce the risk of pathogen contamination. However, many of the intervention methods are less than satisfactory due to limited effectiveness and relatively high costs.

Purpose: The objective of this study was to examine the efficacy of a new sanitizing solution for the inactivation of *E. coli* O157:H7 in selected produce, meat, and poultry products to assure microbial safety and product quality.

Methods: Spinach and lettuce purchased from a local retail market were dip-inoculated, and beef and chicken were spot-inoculated with *E. coli* O157:H7 cells. The beef and chicken samples placed in a stomacher bag containing a mixture of DC-11 and malic acid were hand-massaged (every 30 sec). The produce samples were treated with the same mixture for 1, 2, and 5 min. The samples treated for a specific time period were collected and spread on tryptic soy agar for plate counting.

Results: An increase in the concentration of malic acid did not affect the quality of the samples, while over 1% DC-11 changed the color of the products. A 5-log reduction was achieved on spinach and lettuce when treated with 1% DC-11 and 4% malic acid for 1 min, and the time to achieve a 5-log reduction on the chicken skin was 5 min. The beef treatment obtained a 5-log reduction in 1 min with 0.5% DC-11 and 4% malic acid. Increasing the concentration of malic acid was more effective in reducing the *E. coli* count than increasing the concentration of DC-11. The antibacterial capacity of the DC-11 and organic acid mixtures remained the same in chilled conditions.

Significance: DC-11 and organic acid combined treatments may provide an effective kill step in post-harvest processing operations for securing the microbial safety of produce, meat, and poultry products.

P1-35 The Effects of a CPC Antimicrobial Applied as an Electrostatic Spray on the Shelf Life and Levels of *Escherichia coli* on Raw Beef Briskets

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Introduction: During the hide removal process in beef slaughter, cross contamination of the carcass surface can occur from the initial cut into the carcass beginning at the bung region and extending to the neck of the carcass. As the knife penetrates the hide, bacteria can be transferred from the outside hide area to the sterile underside of the hide.

Purpose: To determine the effects of an electrostatic spray of the cetylpyridinium chloride (CPC) antimicrobial (Cecure® - Safe Foods Corporation) on the natural microflora and inoculated generic *Escherichia coli* on beef surfaces.

Methods: Five brisket samples were cut into two equal pieces, one half for the control and the other half for the CPC antimicrobial spray treatment. Each sample was inoculated with 1,000 generic *Escherichia coli* cells and attached for 30 minutes. Control samples (n=5) did not receive any further treatment. The remaining samples (n=5) were placed on a wire rack and received 133 µg cetylpyridinium chloride/cm^2 (0.4% Cecure®). On Day 0, the control and CPC antimicrobial-treated samples were swabbed (Neutralizing Buffer) using a sterile template (5 x 5 cm^2). The swabbed area was marked with red dye to prevent sampling of the same area on subsequent days. Each sample was then placed on a large sterile plastic tray which was placed in a sterile plastic bag and held at 40°F. Analysis was done using Aerobic Plate Count (APC) and EC Petrifilm™.

Results: Throughout the shelf-life study testing (Day 0-8), treated samples had significantly lower APC and *Escherichia coli* ($P < 0.05$) than the control samples. The control samples reached spoilage levels on Day 4, whereas the treated samples reached spoilage levels on Day 7. The treated samples showed an initial 1 log reduction in the level of *Escherichia coli* compared to the control samples, and thereafter, the level of *Escherichia coli* on both the treated and control samples remained constant throughout the refrigerated storage period. Data were subject to analysis of variance using the general linear model procedure of SAS (1991). Data are presented as natural numbers. Significant differences among or between means were separated by repeated t-tests using the least square means option of SAS software.

Significance: The data show a significant microbial reduction and a significant extension in the shelf-life of beef briskets from the initial reduction in the levels of APC and *Escherichia coli* on beef briskets treated with CPC antimicrobial.

P1-36 Validation of Lactic Acid Interventions on the Reduction of *Escherichia coli* Biotype I, Coliforms and Total Aerobic Bacteria on Chilled Beef Products Processed at a Commercial US Slaughter Facility

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Introduction: Based on previously published studies, lactic acid can reduce *Escherichia coli* O157 and *Salmonella* in beef products. A commercial beef processing plant has selected the use of lactic acid as an intervention for several beef products in their fabrication area as part of a reassessment of their HACCP plan. USDA inspectors requested a validation of this intervention under commercial processing conditions.

Purpose: The purpose of this project was to validate, on site, the use of lactic acid interventions on chilled beef products.

Methods: Chilled carcasses, cheek meat, heart, liver and chilled trim were randomly sampled before/after lactic application (4.2%) at three time points during the day, for a total of three days. A total of 60 samples (30 before and 30 after) were collected during the course of the processing day. All sampling was conducted by in-plant laboratory technicians at the facility and used the N60 sampling method. At the end of the processing day, samples were shipped in cold conditions to the Food Microbiology Laboratory at Texas Tech University for microbial analysis. Generic *E. coli*, coliforms and APC were enumerated for each sample.

Results: All lactic acid interventions for each beef product resulted in significant ($P < .05$) quantifiable reductions in *E. coli* biotype I, coliforms and total aerobic plate counts. The total APC reduction ranged from 0.43 log CFU/cm^2 on cheek product to 2.50 log CFU/cm^2 on carcasses. Coliform reduction was seen from 0.49 log CFU/cm^2 on cheek product to 2.28 log CFU/cm^2 on carcasses. The generic *E. coli* reduction ranged from 1.03 log CFU/cm^2 on cheek product to 3.53 log CFU/cm^2 on carcasses.

Significance: A lactic acid intervention (4.2%) in a commercial processing plant is effective in reducing total microorganisms and microbial indicators on the beef products destined for human consumption. This type of validation is useful for processors to comply with USDA-FSIS regulations and to support HACCP plans and to protect public health.

P1-37 Development of Analytical Method for Lead and Cadmium in Functional Foods and Oriental Medicines Using Standard Addition Method

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Introduction: Oriental medicines and functional foods might be susceptible to contamination with heavy metals such as Pb, Cd, and Hg. Heavy metals have harmful effects on humans and a longer half-life than other organic-contaminated materials. However, heavy metals in oriental medicines and functional foods have been investigated very little.

Purpose: For integrated exposure assessment of heavy metals in Korean functional foods and oriental medicines, the development of a robust analytical method is required. In this study the accurate analytical method for heavy metals in oriental medicines and functional food using ICP-MS was investigated.

Methods: We surveyed functional food and oriental medicine consumption patterns for 2000 Korean people (1000 adults, 1000 students and babies). Samples were categorized by matrix. The levels of Pb and Cd were analyzed with a wet-digestion method using microwave. The operating conditions of the ICP-MS were as follows: Nebulizer (concentric type); R.F. generator (Free-running type, 40 MHz); R.F. power (1400 W); coolant gas flow rate (17.0 L/min); Auxiliary gas flow rate (2.00 L/min).

Results: LOD (limit of detection) of Pb and Cd were 0.4 ng/l and 0.1 ng/l, respectively. LOQ (limit of quantitation) of Pb and Cd were 1.3 ng/l and 0.3 ng/l, respectively. Z-score using CRM (certified reference material) also was less than absolute 1.0.

Significance: The results of this study will be used as a basis for future legislation on the regulation and control of heavy metal contents of oriental medical materials in Korea.

P1-38 Microbiological Risk Assessment for the Application of GAP System on Perilla Leaf and Lettuce

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Introduction: Green perilla leaf and lettuce are one of the plenty of vegetables available in the Republic of Korea. Direct consumption of these raw vegetables after washing may render the possibility of food poisoning outbreaks.

Purpose: This study established risk factors which may cause cross-contamination of food poisoning pathogens in cultivation and harvesting steps in perilla leaf and lettuce farms.

Methods: Samples were collected from cultivation environments and utensils (soil, irrigation water, and sack), plants (perilla leaf or lettuce, stem), personnel hygiene (hand, glove, cloth) and airborne bacteria at three farms (A, B, C) of lettuce and perilla leaf during cultivation and harvesting steps, respectively. The collected samples were assessed for sanitary indications, foodborne pathogens (*Staphylococcus aureus*, *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella* ssp.) and fungi.

Results: As a result, the microbiological risk factors of perilla leaf and lettuce on cultivation and harvest step were confirmed on workers' hands contaminated by unacceptable range while at work. Especially, coliform bacterial population in workers' hands on harvest step at all farms of perillea leaf and lettuce was detected at levels of 3.4~4.4 and 2.9~5.3 log CFU/hand, and *S. aureus* was detected at levels of 1.2~3.4 and 1.5~3.6 log CFU/hand, respectively. *E. coli* O157, *L. monocytogenes* and *Salmonella* spp. were not detected in either of the samples from all farms.

Significance: In conclusion, GAP system should be applied as the way to minimize the microbial risk during cultivation and harvest step of lettuce and perilla leaf as observed in this study.

P1-39 Assessment of Fungal Contamination during Production and Distribution of Rice in Korea

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Introduction: Rice is a staple food in Korea. Recently, it has been reported that the increased contamination of mycotoxin, a secondary metabolite of fungi, was found in rice in South Korea.

Purpose: The purpose of this study is to assess the fungal contamination of rice throughout production, storage and distribution, thus providing better understanding of the potential hazards of fungal contamination in rice.

Methods: Rice samples were obtained from the production and distribution stages. The production stage was divided into 4 different steps:

cultivation, harvesting, storage, and processing (polishing). In the production stage, rice was collected from nine farms which are located in south region of Korea (Jeollanam, Gyeongsangnam and Gyeongsangbuk-do). The rice samples in the distribution stage were obtained from 6 traditional markets as well as 6 local grocery stores in Jinju, Kwangju and Pusan, Korea. The Rose Bengal Agar (RBA) was employed for enumeration of total fungi contamination on the rice sample. In addition, total aflatoxins during distribution stage were determined by HPLC.

Results: The fungal contamination was detected in all samples from production stage at the level of 3.3~4.3 log CFU/g. During the storage period, fungi were detected at high levels of 3.8~5.1 log CFU/g. The level of fungal contamination in the distribution stage was higher in samples from traditional markets than from grocery stores. These were 1.7~3.9 log CFU/g and 1.4~2.9 log CFU/g, respectively. No aflatoxins were detected in the samples from the distribution stage.

Significance: In this study, it was identified that the cross-contamination of fungi on rice can occur during the production and distribution stages. The continuous monitoring of fungal contamination at all stages and the proper application of good agricultural practices (GAP) is strongly recommended in the production and distribution of rice.

P1-40 Levels of Heavy Metal in Meat of Lambs Fed with Crude Glycerin

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Introduction: Substantial expansion of the biodiesel industry has created affordable supplies of crude glycerin. This byproduct has become a major focus in the livestock industry, due to its potential as an alternative energy source in animal feed. However, the presence of heavy metals in crude glycerin is of great concern in food safety and human health due to the risk of contamination in meat.

Purpose: The purpose of this work was to evaluate the level of heavy metals in meat from lambs fed diets with different levels of crude glycerin.

Methods: Thirty non-castrated male lambs were randomly assigned to one of five treatments (0; 3; 6; 9 or 12% of total diet as crude glycerin), with six replicates per treatment. Lambs were confined in individual pens and slaughtered with approximately 35 kg of body weight. From each animal, a 150 g Longissimus dorsi sample was taken for further heavy metal analysis.

Results: It was detected in crude glycerin the presence of (mg/kg): Cu = 5.65; Cr = 1.19; Ni = 5.87; Pb = 0.33; Zn = 5.22 and Cd = level lower than detection limit. Conversely, the elements Pb, Cd, Cr and Ni were not detected in meat samples. There was a linear decrease ($P < 0.05$) of Zn levels in meat with the increase of crude glycerin inclusion in diets. On the other hand, no differences were found ($P > 0.05$) in the levels of Cu among treatments.

Significance: This data suggests that crude glycerin can be used in ruminant diets with levels up to 12% of total diet, since the levels of Zn and Cu are within the tolerable intake levels, without causing toxicity in humans.

P1-41 Species Identification of *Alexandrium minutum* and *A. catenella* by Sequence of 28S rDNA and Cytochrome Oxidase Subunit I Gene

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Introduction: Harmful algal blooms have occurred in temperate aquatic regions throughout the world, negatively affecting aquaculture, fisheries and the tourism industry. Further, HABs pose a risk to human health and the environment.

Purpose: This study attempted to elucidate the sequences of potential species-specific patterns in paralytic shellfish poison (PSP)-producing dinoflagellates (*Alexandrium minutum* and *A. catenella*,) from nuclear and mitochondrial DNA, respectively.

Methods: Three sets of primers used in PCR amplification including one set for 28s rDNA and the other two sets for mitochondrial cytochrome oxidase subunit I gene. The PCR products were validated by agarose gel electrophoresis, and sequenced by an ABI DNA sequencer.

Results: The crude genomic DNA yielded an apparent band (>3000 bp) from two species of *Alexandrium*. The electrophoretic analyses of the PCR products of the 28S r-DNA region from *A. minutum* and *A. catenella* exhibited the 688 and 684 bp fragments, respectively. Comparison of the similarity of the sequences with the gene bank (Accession number AF033532 and AF019408), showed that similarity was 100% for *A. minutum* and 99.6% for *A. catenella*. According to their sequence data of nuclear 28S r-DNA, *A. minutum* and *A. catenella* could be differentiated. Mitochondrial cytochrome oxidase subunit 1 gene (COX1) of dinoflagellate *Alexandrium* species were first identified in this paper by two sets of primers, COD and COP. It showed the 644 bp and 398 bp PCR products amplified from COD and COP primers, respectively. The COX1 gene fragments of these two *Alexandrium* species were sequenced; however, these two fragments cannot be distinguished from each other.

Significance: In this study, though the morphologies of *A. minutum* and *A. catenella* are quite similar, the sequence data of 28S r-DNA are significantly different.

P1-42 Effect of Lactose on the Biological Activity of Ricin

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Introduction: Lactose, a major component of milk, is a natural inhibitor of ricin. However, it is unknown if the level of lactose found in milk is enough to protect against ricin intoxication.

Purpose: To evaluate the effect of lactose (at the typical concentration in milk) on the ability of ricin to inhibit protein synthesis in HeLa (human) cells.

Methods: Ricin samples (0.001-300 µg/mL) treated with/without 134 mM lactose (1 mL) were added to test tubes containing 1 mL HeLa cells (3×10^5 cells in a low leucine medium). After 2 h of incubation at 37°C, 0.5 µCi of L-[U-14C]-leucine was added to each tube, followed by 60 min of further incubation. The tubes were centrifuged (1000 x g for 10 min), and the cell pellet re-suspended in 1 mL of 0.1 N KOH for 30 min. The solutions were then made up to contain 10% (w/v) trichloroacetic acid, followed by centrifugation (13,000 x g for 10 min) to precipitate the proteins on the walls of the tubes. The amount of radioisotope incorporated into cellular proteins was determined by liquid scintillation. Biological activity was defined as the amount of radioactivity in a sample relative to that of the control (cells not treated with ricin).

Results: The effect of 134 mM lactose on the biological activity of ricin was only significant at concentrations of ricin below 1 µg/mL. At 1 ng/ml of ricin, inhibition of protein synthesis in the presence of lactose was 20%, whereas inhibition was 88.5% in its absence. At 100 ng/ml, inhibition of protein synthesis was 76.4% in the presence of lactose, and 94.1% in its absence.

Significance: These data suggest that milk lactose may not protect against ricin at the concentration (6.22 µg/mL) equivalent to its LD50 for a 20 kg child consuming 225 mL of milk.

P1-43 Use of a Dry Steam Belt Washer for Removal of Allergenic Food Residue

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Introduction: Validated cleaning procedures are essential for preventing allergen cross-contact from shared processing equipment. Dry cleaning methods, such as those involving the use of dry steam, have been developed for removing allergenic food residues from food-contact surfaces. However, little is known about the effectiveness of these methods.

Purpose: The objective of this study was to validate the effectiveness of a commercially available belt washing system that uses dry steam in combination with vacuum for removing allergenic food from a urethane-faced conveyor belt.

Methods: Undiluted peanut butter was applied and slurries of non-fat milk (10,000 µg/mL) and egg powder (24,000 µg/mL) were pipetted onto a urethane-faced conveyor belt. The belt was cleaned using a commercially available dry steam belt cleaning unit for 10 min. At two min intervals, the belt washer was stopped, and the belt was swabbed using conventional ATP, sensitive ATP, total protein and allergen-specific lateral flow tests (ELISA) to determine the presence of allergenic food residue. All cleaning trials were done in triplicate and at least three swabs were obtained for each analytical method.

Results: Sensitive ATP swabs of the conveyor belt showed high levels of ATP for peanut, milk and egg no matter how long the belt was cleaned. However, for most trials with the three different food residues, conventional ATP swabs indicated the absence of ATP after 10 min of cleaning. Total protein swabs detected all three types of food residue on the belting after cleaning for 10 min. No egg residue was detected with the lateral flow tests for all the cleaning times tested, while peanut and milk residue was still detected after cleaning the belt for 10 min.

Significance: This study demonstrates that the efficacy of the dry steam cleaning unit depends on the type of food soil applied to the belt surface. Care must be taken when choosing a method for detecting the presence of food residues in a dry cleaning system.

P1-44 Evaluation of Commercial ELISA Test Kits for Detection of Milk Proteins in Cooking Oil

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Introduction: Milk is a common ingredient in fried food. Allergen cross contact can occur through the use of shared frying oil. Analytical methods are needed to determine the level of protein contamination in re-used oil.

Purpose: This study evaluated the performance of four ELISA test kits in comparison with a total protein assay for detection of milk protein residues in spiked oils that have been subjected to frying at different temperatures.

Methods: NIST non-fat milk powder standard reference material #1549 and Backpacker's Pantry powdered whole milk were heated in Mazola® corn oil at 150°C or 180°C for 3 minutes in a DeLonghi Cool-Touch® deep fryer. The amount of milk proteins in the oil samples were determined using the Pierce 660 nm total protein assay as well as four commercial ELISA kits (Veratox for Total Milk Allergen, BioKits BLG Assay Kits, ELISA SYSTEMS Casein and Beta-Lactoglobulin Residue assays) either directly or after extraction with phosphate-buffered saline with 0.05% Tween (PBST) followed by partitioning in hexane to remove residual oil.

Results: Based on the Pierce total protein assay, PBST/hexane extraction resulted in a 90% or 80% recovery of milk proteins from unheated oils spiked with non-fat or whole powdered milk, respectively. Frying at 150°C caused an approx. 75% decrease in the amount of protein recovered from both the non-fat and whole milk samples, while frying at 180°C resulted in a > 90% decrease in protein recovered. All four ELISA kits were able to accurately determine the amount of milk residues in unheated oil samples. However, frying at 150°C or 180°C resulted in a significantly lower amount of protein detected by these kits. Certain test kits registered greater protein levels than those detected by other test kits or the Pierce total protein assay. For example, the relative amount of protein in non-fat milk fried at 150°C as determined by the BioKits BLG test was more than 2 or 10 folds greater than those measured by the Pierce assay or the ELISA SYSTEMS BLG kit, respectively. Inclusion of the PBST/hexane extraction step prior to test kit analyses may or may not result in a greater amount of protein detected, depending on the test kits used.

Significance: Commercial ELISA test kits are capable of detecting milk residues in cooking oils; however, the accuracy of quantitation was dependent on the test kits and the frying conditions used.

P1-45 Variation in Microbial Communities among Abattoirs and between Primal Cuts on Refrigerated Vacuum-packed Beef

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Introduction: Vacuum-packaged Australian primals are known to have an enhanced shelf life in export markets. However, the basis for this condition is not well understood. It is known that the shelf life of fresh beef is influenced by temperature, pH, packaging atmosphere and the effects of certain microbial species. However, relatively little is known about how complex bacterial communities influence shelf life.

Purpose: The purpose of this study was to define microbial communities on striploin and cube roll processed at six abattoirs and stored over 30 weeks at -0.5°C, and to determine how communities vary among abattoirs and on different meat types.

Methods: Striploins and cube rolls were collected from six export abattoirs and stored at -0.5°C for 30 weeks. At selected time intervals, DNA was extracted from meat rinsates. Bacterial community analysis was performed using terminal restriction fragment length polymorphism (tRFLP) and clone libraries of the 16s rRNA gene. Differences among tRFLP profiles were determined by an analysis-of-similarity test using Primer6 software. For clone libraries, sequences were aligned using BioEdit and statistical differences determined with the UniFrac program.

Results: Microbial communities were different among the six abattoirs, and between striploin and cube roll, by tRFLP analysis. Clone libraries showed, using a subset of four abattoirs representing high and low Total Viable Count and Lactic Acid Bacteria growth rates, that bacterial species were significantly different among the abattoirs during 30 weeks of storage. Analysis of striploin versus cube roll showed that differences in bacterial species between meat types increased with storage time.

Significance: These findings demonstrate that bacterial communities on fresh beef are complex and that as yet undefined bacterial and environmental factors likely influence community structure. Understanding these interactions could assist with the design of meat process operations that lead to enhanced shelf life.

P1-46 Determination of Risk Factors Associated with *Salmonella* and *Escherichia coli* O157 Prevalence on Carcasses in a Mexican Slaughter Plant

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Introduction: During 1993 - 2002, six hundred thirty-three foodborne outbreaks were reported in Mexico, with 12,748 people being affected, primarily with *Salmonella, Escherichia coli* and *Shigella*. Consequently, the identification of sources of *Salmonella* and *E. coli* O157 in a slaughter plant is essential to identify methods for reducing the prevalence of these pathogens on carcasses and therefore, reduce the risk of foodborne diseases to the consumers.

Purpose: To identify risk factors associated with detection of *Salmonella* and *E. coli* O157 on carcasses in a slaughter plant

Methods: Two hundred fifty animals were randomly selected and composite samples were taken from three anatomical carcass sites (inside round, hind shank and fore shank). Samples were taken from the hides, fecal grabs (FG), at pre-evisceration (PE), prior to entering the hot box (PHB) and after 24 hours of dry chilling (DC). Additionally, 250 samples from fecal (FL) and water samples were taken from the lairage area. *E. coli* O157:H7 and *Salmonella* detection was carried out using BAX, IMS and conventional methods. Logistic regression models were used to determine areas of highest risk.

Results: Due to low prevalence of *E. coli* O157:H7, risk factors were not detected. Animals positive for *Salmonella* in FG had an 8 times higher chance to test positive on hides ($P = 0.006$), 3 times more likely to test positive on carcasses at PE ($P = 0.0001$), and were 2 times more likely to test positive on carcasses at PHB ($P = 0.01$). Moreover, positive carcasses at PE increased the chance of obtaining a positive carcass test at PHB by 6 times ($P = 0.0001$); and positive carcasses at PHB increased the chance of a positive carcasses at DC ($P = 0.003$) by 8 fold.

Significance: FG is an important determinant factor in the detection of *Salmonella* in hides and carcasses at slaughter plants. Implementation of pre-harvest controls is recommended to reduce the risk.

P1-47 Comparison of *Salmonella* and *Escherichia coli* O157 Prevalence on Beef Carcasses Harvested in Mexico under Two Different Production Procedures under TIF Regulations

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Introduction: Federal Inspection Type (TIF) standards in Mexico ensure the safety of meat products prepared in establishments holding the TIF certification. TIF slaughterhouses comply with national and international food safety specifications; however, they have differences in the production procedures and interventions depending of the final market. Supermarkets require interventions such as hot water and lactic acid application, while other markets such as wet markets, street vendors and butcher shops don't.

Purpose: To compare the prevalence of *Salmonella* and *E. coli* O157:H7 on carcasses destined for different markets processed under TIF certification in Mexico.

Methods: A total of 120 animals were sampled in the plant at the following locations: fecal grabs (FG), hides, at pre-evisceration (PE), prior entering the hot box (PHB) and after 24 h of dry chilling (DC). The first group of carcasses was destined for supermarkets (DSM) and received a lactic acid spray of 2.1% (25°C) at PHB. A second group of animals, destined for smaller market venues (DMV), were sampled at the same locations and processing days as the first group; but their carcasses did not receive microbiological interventions. *E. coli* O157:H7 and *Salmonella* detection was carried out using BAX, IMS and conventional methods

Results: The *Salmonella* prevalence in FG was higher (26%) in cattle DSM, than those DMV (10%); however, the latter had higher detection on hides and at PE (100% and 35%; respectively) compared to those DSM (82% and 14%; respectively). Both groups had no differences ($P > 0.05$) in *Salmonella* prevalence at PHB (5%) and DC (0%). The *E. coli* O157:H7 prevalence did not differ between DSM and DMV ($P > 0.05$) in FG (DSM 11% vs. DMV 5%), hide (DSM 6% vs. DMV 5%) and carcasses (0%).

Significance: While the DMV carcasses were not subjected to an intervention, the pathogen prevalence was low in this TIF facility due to careful dressing procedures

P1-48 *Salmonella, Campylobacter* and Putative Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) in Ground Beef and Whole Muscle Beef Cuts in the United States

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Introduction: Non-O157 STEC, *Salmonella*, and *Campylobacter* cause considerable human illnesses each year and the vast majority of cases are foodborne. Currently, better understanding of the burden of these pathogens in the U.S. is of much interest.

Purpose: To establish an estimate or prevalence of *Campylobacter*, *Salmonella*, and Non-O157 STEC in beef products collected from U.S. retail markets.

Methods: Thirty-two American cities were sampled in this study. Retail raw ground and whole muscle beef (n=2,915) samples were purchased and examined for the presence of *Salmonella* using a PCR assay. Of the original samples purchased, n=1,211 were randomly selected and tested for *Campylobacter*. Positive samples were confirmed through direct plating and agglutination. Samples were enriched using Bolton Broth and grown on blood-free *Campylobacter* plates in required microaerophilic conditions. A sub-sample (n=325) was screened for putative non-O157 STEC using rtPCR methods.

Results: Salmonella was detected in 0.65% of the total samples purchased (n=19). *Campylobacter* was recovered from 9.3% of samples whereas putative non-O157 STEC antigens were detected in 5.9% of samples. The most common serotypes detected in this study were O26 (3.8%), O145 (2.2%), O103 (1.3%), and O111 (0.98%).

Significance: Creating pathogen baselines in U.S. retail beef is imperative for targeting interventions for pathogen control. These data can be utilized for a more complete understanding of these pathogens and their impact on public health from the consumption of beef products.

P1-49 Prevalence Study of Top Six Non-O157 STEC in Raw Beef in the United States

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Introduction: As a result of recent incidents of contamination, there is growing interest in the United States in the potential public health risk associated with a broader group of *E. coli* commonly referred to as "the top six non-O157 shiga toxigenic *E. coli*" or "Top STEC." Most surveillance studies of clinical isolates report that the following six STEC O serotypes, in addition to O157, represent the greatest public health risk: O26, O45, O103, O111, O121 and O145.

Purpose: Little data for the prevalence of the Top STEC microorganisms exists in raw beef.

Methods: A surveillance study was undertaken to look at the incidence of Top STEC from raw beef samples. Five beef processors located in various parts of United States evaluated 1800 beef trim and ground beef samples with Assurance GDS® Top STEC and Shiga Toxins for Top STEC assays.

Results: Sixty-three *E. coli* isolates were identified as containing one of the top six serotypes and one of the two targets established by the USDA that define the Top STEC. Among these isolates, 16 contained both targets. At least one Top STEC from each serogroup was isolated.

Significance: These data provide insight into the potential incidence rate of Top STEC in raw beef in the United States.

P1-50 Validation of Hot Water and 5% Lactic Acid as an Intervention to Reduce *Escherichia coli* O157:H7 on Chilled Beef Trim

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Introduction: The CDC reports that there are an estimated 70,000 *E. coli* O157:H7 infections in the US annually, with many originating from beef. Research has examined the use of 5% lactic acid and hot water (180°F) to reduce *E. coli* O157:H7 on hot carcasses, but little data exist on the efficacy of these interventions on chilled surfaces.

Purpose: The purpose of this study was to determine if applying 5% lactic acid and hot water (180°F) to chilled beef trim would result in a reduction of *E. coli* O157:H7 and indicator organisms.

Methods: Beef trim was inoculated with a cocktail of *E. coli* O157:H7 (10^5 CFU/ml). Inoculated chilled trim was treated for 1 min by spraying with 5% lactic acid, ambient water, or by dipping in hot water (180°F) for 1 min. The total *E. coli* O157:H7, generic *E. coli* and total aerobic plate counts were determined by direct plating.

Results: All three treatments reduced the total aerobic bacteria on the meat from 4.67 log CFU/25 g in the control to 4.07 log CFU/25 g, 3.58 log CFU/25 g and 3.52 log CFU/25 g in trim treated with water, hot water and 5% lactic acid, respectively. The generic *E. coli* were reduced from 4.32 log CFU/25 g in the control to 3.31 and 3.38 log CFU/25 g for 5% lactic acid and hot water, respectively. Ambient water had little effect. *E. coli* O157:H7 was reduced significantly with the 5% lactic acid with populations declining from 3.92 bacteria/25 g in the control to 3.19 total bacteria/25 g. Ambient and hot water did not reduce the total *E. coli* O157:H7.

Significance: Results can be used to validate the use of lactic acid to reduce pathogens on chilled beef trim and provide evidence that this intervention can be effective in reducing the potential of a recall.

P1-51 Low-Energy X-Ray Irradiation against *Escherichia coli* O157:H7 in Ground Beef of Different Fat Contents and Product Temperatures

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Introduction: Contamination of ground beef with *Escherichia coli* O157:H7 remains an ongoing problem as evidenced by continued outbreaks of illness and the recall of ~1.5 million lbs. of ground beef in 2010. Low-energy X-ray irradiation is a potentially effective means of reducing the risk, but there is a lack of information related to the impact of product characteristics on microbial efficacy.

Purpose: The objective of this study was to quantify the microbial efficacy of low-energy x-ray irradiation against *E. coli* O157:H7 in ground beef of two different fat contents and product temperatures, and to assess the performance of a predictive model.

Methods: Low (4.9%) and high (16.6%) fat ground beef were inoculated with a four-strain cocktail of *E. coli* O157:H7. Inoculated samples (~5 g, ~7.7 $\log_{10}$ CFU/g) were pressed thin (3 mm) in a plastic bag to ensure a uniform X-ray dose, and then the samples were stored at -23°C (~26 days). Half of the samples were then equilibrated to 4°C overnight, while half remained at -23°C. Thereafter, the inoculated and temperature-equilibrated samples were irradiated (5 doses, in triplicate, ~0.5 kGy) using a prototype X-ray irradiator (70 kV). All irradiated samples were homogenized in phosphate buffer, appropriately diluted and then plated in duplicate on Sorbitol MacConkey Agar containing cefixime and tellurite (18–24 h; 37°C), to enumerate survivors. In addition, X-ray free-field analysis was coupled with a density mapping of the product, which was reconstructed from computer tomography images, to develop a lethality predictive model.

Results: For frozen samples, X-ray D_{10}-values were 0.10 kGy and 0.14 kGy for high fat and low fat sample, respectively. The D_{10}-values for refrigerated samples were ranging 0.051 to 0.054 kGy. Microbial inactivation was significantly different between frozen and refrigerated samples ($P < 0.05$). However, there was no significant difference between high and low fat ground beef in refrigerated state ($P > 0.05$), but significant in frozen patties ($P < 0.05$). The error of the dose/lethality prediction model was ranging 20 to 38%.

Significance: Based on the findings to-date, *E. coli* O157:H7 on ground beef can be effectively eliminated using low-energy X-ray irradiation. Additionally, an integrated process model encompassing biological and physical models can be developed to assess the feasibility of this technology.

P1-52 Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Beef Trim

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Introduction: Escherichia coli O157:H7 and *Salmonella* are major causes of foodborne disease.

Purpose: The purpose of this study was to evaluate the efficacy of a levulinic acid and sodium dodecyl sulfate treatment as a surface application to reduce *E. coli* O157:H7 and *Salmonella* populations on beef trim.

Methods: Levulinic acid plus SDS at different concentrations, temperatures, contact times, and agitation speeds was evaluated for the inactivation of *E. coli* O157:H7 and *S.* Typhimurium on beef trim surfaces at different meat temperatures. Beef (25–35% fat content) was cut into 10 x 10 x 7.5-cm portions and inoculated with *E. coli* O157:H7 or *S.* Typhimurium at 10^7 CFU/cm^2. The relationship between chemical treatment conditions and beef temperatures was determined.

Results: Results revealed that the bactericidal effect of 3% levulinic acid plus 2% SDS (LV/SDS) solution was related to beef surface temperature. Treating 5°C-beef trim with LV/SDS at 21°C, 62°C, or 81°C for 30 sec reduced *E. coli* O157:H7 by 1.0, 1.1, or 1.4 log CFU/cm^2, respectively, whereas treating 8°C-beef trim with LV/SDS at 12°C for 0.1, 1, 3, or 5 min reduced *E. coli* O157:H7 by 1.4, 2.4, 2.5, or 3.3 log CFU/cm^2, respectively. Similar results were obtained with *S.* Typhimurium. Treating 8°C-beef trim with LV/SDS for 1, 2, or 3 min reduced *S.* Typhimurium by 2.1, 2.6, and >5.0 log CFU/cm^2, respectively. Applying hand massage to the treated beef trim substantially increased the reduction of both pathogens, with no detectable *E. coli* O157:H7 or *S.* Typhimurium (< 5 CFU/cm^2) on 12°C-trim treated with LV/SDS at 12°C for 30 sec compared to 3 log CFU/cm^2 present on the water-treated control.

Significance: The LV/SDS treatment was effective in reducing *E. coli* O157:H7 and *S.* Typhimurium populations by >3 log CFU/cm^2 on beef at 8°C, but the bactericidal activity was closely related to the meat temperature.

P1-53 The Effects of Salt, Sodium Pyrophosphate and Sodium Lactate on the Growth Behavior of *Escherichia coli* O157:H7 in Ground Beef

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Introduction: Ground beef products have been linked to outbreaks of foodborne illness causing by *Escherichia coli* O157:H7.

Purpose: This study examined the effect of salt (0 –2.25%), sodium pyrophosphate (SPP, 0 –0.5%), and sodium lactate (0 –3%) on the growth behavior of *E. coli* O157:H7 in ground beef during temperature-abuse storage.

Poster

Methods: Ground beef (3 g) samples containing 25 combinations of the three additives were inoculated with a 4-strain cocktail of *E. coli* O157:H7 to an inoculum of 10^3 CFU/g, vacuum-packaged, and stored at 10°C for 15 days. Twenty samples for each combination were tested to obtain the growth probability of *E. coli* O157:H7. A sample was considered as able to support growth of *E. coli* O157:H7 when the count at the end of the storage was 1.0 log CFU/g higher than the initial inoculum. The growth probabilities *(P)* as a function of salt, SPP, and lactate were analyzed with a logistic regression.

Results: Growth ($P \geq 0.5$), uncertainty of growth ($0.1 < P < 0.5$), and no growth ($P \leq 0.1$) of *E. coli* O157:H7 predicted by the resulting probability model were in agreement with those experimentally observed, indicating that the model closely described the behavior of *E. coli* O157:H7 in ground beef. Salt and lactate exhibited substantial effect on the growth probability of *E. coli* O157:H7, whereas the effect of SPP was marginal.

Significance: Results from this study will help in identifying the levels of these three additives in ground beef products to control the growth of *E. coli* O157:H7.

P1-54 Effect of Household Storage, Thawing and Cooking Practices on the Survival of *Salmonella* and *Escherichia coli* in Beef Patties

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Introduction: Food Safety authorities worldwide have issued general guidelines for the proper handling of ground beef in households. However, these recommendations may not sufficiently cover all the commonly applied practices, because the latter depend on the convenience and personal preferences of the consumers for handling and consuming foods.

Purpose: The objective of the study was to evaluate the survival of *Salmonella* and *Escherichia coli* O157:H7 during frozen storage, thawing and cooking of beef patties, simulating common consumer-style practices.

Methods: Portions (400 g) of ground beef were inoculated (~6.5 log CFU/g) with a five-strain composite of *Salmonella* or a three-strain composite of *E. coli* O157:H7 and stored at -22°C. After 5 and 75 days of frozen storage, thawing took place (i) in refrigerator at 4°C for 16 hours; (ii) at 20°C for 12 hours, simulating thawing on counter, or; (iii) in microwave for 22–24 minutes. Following thawing, 90 g beef patties were shaped and cooked in Double-Broil (DB) or Pan-Grill (PG) until the internal temperature reached 60°C (simulating undercooking) or 71°C (recommended cooking temperature). In addition, the survival of the two pathogens after direct cooking of frozen patties was studied, simulating commercial cooking practices in catering services and restaurants.

Results: Cooking in DB was more effective for the elimination of the pathogens compared with cooking in PG, especially when cooked at 71°C. These findings showed that the survival of the pathogens was highly affected by the rate and the direction of heat transfer into the beef patties, during cooking, regardless of the thawing method applied before. *E. coli* O157:H7 showed an increased survival during cooking after frozen storage for 75 days, compared to 5 days of storage, while the opposite was observed for *Salmonella*.

Significance: The results may be used to instruct the consumers and catering services for the appropriate defrosting or cooking practices of beef patties, taking into account the variability that might occur for practical purposes in different establishments (i.e., households, food caterings, restaurants).

P1-55 Inactivation of Non-O157:H7 Shiga Toxin-producing *Escherichia coli* (STEC) in Frozen Ground Beef Patties

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Introduction: A cluster of illnesses linked to contamination of ground beef with an *E. coli* serotype O26 strain, and the subsequent recall, reinforces the need for additional research on control of STEC in beef.

Purpose: Quantify the effect of cooking on the fate of STEC in refrigerated and frozen ground beef patties of both high and low fat content when cooked on commercial grills.

Methods: Ground beef (percent lean:fat = 70:30 and 93:7) was inoculated with about 7.0 log CFU/g of a serotype O26, O45, O103:H2, O121, O145:HNM, and O111:H7 cocktail of STEC. Ground beef patties (ca. 300 g, ca 2.54 cm thick) were formed, stored at 4°C for 18 hours (i.e., refrigerated) or at -20°C for 21 days (i.e., frozen), and then cooked on either an open flame gas grill or on an electric clam-shell grill to target internal temperatures of either 60.0°, 65.5°, 71.1°, or 76.6°C.

Results: Regardless of the level of fat or type of grill, cooking refrigerated patties to 71.1° or 76.6°C resulted in a ≥6.2 log CFU/g decrease in STEC, with decreases of 3.2 to 5.1 and 5.7 to 6.4 log CFU/g at 60.0° and 65.5°C, respectively. When frozen patties were cooked to 65.5°, 71.1°, or 76.6°C, regardless of the fat level or type of grill, pathogen numbers decreased by 4.7 to 6.0 log CFU/g, with a decrease of 2.5 to 4.4 log CFU/g after cooking to 60.0°C.

Significance: These data confirm that despite non-uniform heating of patties, cooking refrigerated or frozen ground beef patties to the recommended internal temperature of 71.1°C is sufficient to lessen consumer exposure to any STEC present.

P1-56 Thermal Inactivation of *Escherichia coli* O157:H7 (ECOH) in Frozen Ground Beef Patties Following Cooking on Commercial Open-flame Gas and Electric Clam-shell Grills

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Introduction: Both the prevalence and levels of serotype O157:H7 strains of *Escherichia coli* (ECOH) are quite low, yet this pathogen continues to cause foodborne illness due to consumption of undercooked ground/non-intact beef. Thus, further studies are warranted to comparatively quantify thermal destruction of ECOH in refrigerated versus frozen ground beef patties.

Purpose: Quantify the effect of both fat and grill type on the fate of ECOH in refrigerated and frozen ground beef patties during cooking.

Methods: Both high fat and low fat ground beef (percent lean:fat = 70:30 and 93:7, respectively) were purchased from a local butcher and inoculated with a 5-strain cocktail of ECOH (ca. 7.0 CFU/g). Patties were pressed (ca. 2.54 cm thick, ca. 300 grams) and then either refrigerated (4°C, 18 hours) or frozen (-20°C, 3 weeks) before being cooked on a commercial open-flame gas grill or on a clam-shell electric grill to internal temperatures of 60° to 76.6°C.

Results: Regardless of the type of grill, cooking low and high fat refrigerated patties to 71.1° or 76.6°C decreased ECOH numbers from ca. 7.2 log CFU/g to ≤0.9 log CFU/g, whereas decreases from ca. 7.2 log CFU/g to ca. 1.0 to 3.0 log CFU/g in pathogen numbers were observed when refrigerated patties were cooked at 60.0° or 65.5°C. For low and high fat frozen patties that were cooked to 71.1° or 76.6°C, ECOH

numbers decreased from ca. 6.7 log CFU/g to ca. 0.6 to 1.5 log CFU/g. Likewise, pathogen numbers decreased from ca. 6.7 log CFU/g to ca. 1.7 to 3.3 log CFU/g when frozen patties were cooked to 60.0° or 65.5°C.

Significance: Although we observed non-uniform heating of patties, these results validated that cooking refrigerated and frozen ground beef patties to the recommended internal temperature of ≥71.1°C is effective for destroying ECOH and, in turn, lessening the threat of illness associated with this foodborne pathogen.

P1-57 Fate of *Escherichia coli* O157:H7 (ECOH) in Blade Tenderized Beef Prime Rib Following Searing, Cooking and Holding under Commercial Conditions

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Introduction: Undercooked non-intact beef has caused a number of illnesses due to contamination with serotype O157:H7 strains of *Escherichia coli* (ECOH). Only a few studies have quantified translocation and/or thermal inactivation of ECOH directly in blade tenderized beef. However, there have been no such studies for prime rib, yet this product may pose a greater risk to public health than others because it is cooked/held for extended times at lower temperatures.

Purpose: Evaluate the effect commercial searing, cooking, and holding times/temperatures for elimination of ECOH within blade tenderized beef prime rib.

Methods: Boneless prime rib was inoculated on the fat side with ca. 6.0 log CFU/g of a five-strain cocktail of ECOH and then passed once through a blade tenderizer with the fat side facing upwards. The tenderized prime rib was seared at 500°F for 30 min in a conventional (home) oven and then cooked in a commercial convection oven at 250°F to internal temperatures of 100°, 120°, 140°, and 160°F before being placed in a commercial holding oven (140°F) for up to 8 hours. At each sampling interval, two 1.5-inch thick steaks were cut from each piece of prime rib to be sampled and separately macerated and subsequently surface plated onto sorbitol MacConkey agar plates containing rifampicin (100 µg/ml; SMACR).

Results: After searing, ECOH numbers decreased by ca. 1.2 log CFU/g. Following cooking to 100°F pathogen numbers decreased by an additional ca. 1.3 log CFU/g, whereas cooking to internal temperatures of 120°, 140°, and 160°F resulted in an additional decrease of ca. ≥2.4 log CFU/g. After cooking to 100°F and then holding at 140°F for 8 hours, pathogen numbers increased by an additional ca. 1.3 log CFU/g. In contrast, after cooking to 120°, 140°, or 160°F and holding for up to 8 hours at 140°F, ECOH numbers remained relatively unchanged.

Significance: The commercial practices for searing, cooking, and holding prime rib evaluated herein are effective for inactivating cells of ECOH that may exist within the deeper tissues of the meat due to blade tenderization if the product is cooked to temperatures ≥120°F.

P1-58 Thermal Inactivation of *Escherichia coli* O157:H7 on the Surface or within Non-intact Steaks as Affected by Thickness, Thawing, Starting Cook Temperature and Cooking Method

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Introduction: Escherichia coli O157:H7 transferred into internal muscle tissue during blade tenderization or moisture enhancement is a potential cause of foodborne illness if the meat is undercooked, and current pathogen lethality guidelines only indicate time and temperature as parameters to ensure the safe consumption of beef products.

Purpose: This study evaluated the effect of steak thickness, thawing from the frozen state and the subsequent starting cook temperature, and cooking method on the thermal inactivation of *E. coli* O157:H7 on the surface or within blade-tenderized non-intact steaks cooked to a sublethal temperature.

Methods: Fresh beef (*Semitendinosus*; 3% fat) was cut into 0.6 cm thick slices, and two or four of these slices were stacked on top of each other to form 1.2 or 2.4 cm thick steaks, respectively. The steaks were blade-tenderized with a hand-held tenderizer (4.8 blades/cm^2), inoculated with rifampicin-resistant *E. coli* O157:H7 (8 strains; 3.8 ± 0.4 log CFU/cm^2) on the external surface or between the slices, vacuum-packaged, and stored at -20°C (5 days). Steaks were cooked at 149°C, directly from the frozen state (-20°C) or after thawing to 4 or 25°C, by pan-broiling or roasting, to a steak geometric center temperature of 60°C. After cooking, a core sample (1.61 cm^2) was excised from the center of each steak and analyzed (two repetitions, three samples/treatment/repetition) for *E. coli* O157:H7 (tryptic soy agar plus 0.1% sodium pyruvate and 100 µg/ml rifampicin). Main effects were analyzed using a full factorial design in Proc Mixed of SAS and treatment means were separated using an *F*-protected t-test.

Results: The temperature of steaks at the start of cooking [frozen (-20°C), thawed (4, 25°C)] did not ($P \geq 0.05$) affect surviving pathogen counts in cooked (60°C) samples. For pan-broiled steaks inoculated in the geometric center, *E. coli* O157:H7 counts recovered were 2.7 ± 0.2 to 3.3 ± 0.2 log CFU/cm^2, regardless of steak thickness; however, for samples inoculated on the external surface, fewer ($P < 0.05$) pathogen cells were recovered from 2.4 cm (0.9 ± 0.5 to 1.3 ± 0.6 log CFU/cm^2) than from 1.2 cm (2.1 ± 0.6 to 2.3 ± 0.3 log CFU/cm^2) thick steaks, after cooking. For steaks cooked by roasting, no such trends were found, and surviving pathogen counts ranged from 2.3 ± 1.3 to 3.4 ± 0.3 log CFU/cm^2 in all samples. In general, higher numbers of pathogen survivors were obtained in steaks cooked by roasting than by pan-broiling.

Significance: These data suggest that steak thickness and cooking method, but not starting cooking temperature after thawing, should be considered in lethality guidelines developed to ensure the safe consumption of non-intact meat products.

P1-59 Reduction of *Escherichia coli* O157:H7 in Needle-tenderized Beef Strip Steaks Using Lactic Acid and Cooking

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Introduction: Mechanical tenderization improves beef palatability. However, mechanical tenderization increases risk of pathogen translocation to the interior of beef cuts and foodborne illnesses have been associated with the consumption of non-intact beef products.

Purpose: This study investigated the efficacy of lactic acid spray (5%; LA), on the survivability of *Escherichia* O157:H7 in mechanically tenderized beef steaks managed under simulated industry conditions and cooked to various degrees of doneness.

Methods: Beef strip loins inoculated with high (10^5 log CFU/cm^2; HI) or low (10^3 log CFU/cm^2; LOW) amounts of *E. coli* O157:H7 were treated (5% LA or control), vacuum packaged, and stored for 21 d (0–4°C). After 21 d, subprimals were mechanically tenderized and portioned into steaks, followed by storage for an additional 7 d (2 to 4°C). Twenty-eight d post-inoculation, steaks were cooked to five internal temperatures: 55, 60, 65, 70, and 75°C. Microbial samples were taken from subprimals: fifteen minutes post-inoculation, after LA treatment, and prior to tenderization (d 21). Surface swabs were taken from steaks before cooking and five minutes after reaching internal temperature. Surface swabs were enumerated by plating on MacConkey's agar with TSA overlay. Core samples from cooked steaks were evaluated for *E. coli* O157:H7 presence using DNA-based detection methods.

Results: Treatment with 5% LA reduced initial (0 d) levels of *E. coli* O157:H7 on lean and fat surfaces of HI and LOW subprimals ($P < 0.05$). After 21 d of storage, LA did not affect the total *E. coli* O157:H7 on lean surfaces of HI subprimals ($P > 0.05$); however, a 1.0 log CFU/cm^2 reduction was observed on fat surfaces ($P < 0.05$). No treatment effect was observed on either lean or fat surfaces on LOW subprimals after 21 d of storage. Lactic acid spray reduced ($P < 0.05$) *E. coli* O157:H7 on the surface of steaks from mechanically tenderized HI subprimals compared to controls. However, *E. coli* O157:H7 was detected in core samples obtained from high inoculated, needle tenderized steaks cooked to 55, 60, or 70°C. Presence in cooked samples was lower than in raw samples ($P < 0.05$). *Escherichia coli* O157:H7 was not detected in core samples obtained from LOW steaks, regardless of internal cook temperature; however, 25% of raw samples were positive for *E. coli* O157:H7.

Significance: These data suggest treatment with 5% LA spray reduces *E. coli* O157:H7 in mechanically tenderized steaks subjected to industry storage conditions prior to cooking to 55°C or higher internal temperatures.

P1-60 Survival of *Salmonella* spp. during Preparation of Blade-tenderized, Rare, Beef Prime Rib

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Introduction: Beef prime rib is a delicacy that is traditionally cooked to low temperatures and held for long periods of time. Blade tenderization and other processes used in meat processing may cause translocation of surface pathogens into the interior and may allow for survival and potential growth of foodborne pathogens, if present, in the product during cooking and holding operations.

Purpose: Evaluate the destruction, survival, and/or growth of *Salmonella* spp. during preparation of prime rib from non-intact beef.

Methods: Beef rib eye was surface inoculated with a five-strain cocktail of *Salmonella* spp. to obtain ca. 6.0 log CFU/g. The ribeye was tenderized by passing the fat-side inoculated ribeye through the blade tenderizer with the fat side facing upwards. The ribeye was seared for 15 min at 500°F, cooked in a commercial oven at 250°F to internal temperatures of either 100° or 120°F and held at 140°F for up to 8 h. Slices (1 inch portions from each end) of a prime rib were obtained after searing and cooking, as well as during holding for 2, 4, 6 and 8 h.

Results: Searing of blade-tenderized rib eye resulted in 0.55 log CFU/g reductions in levels of *Salmonella*. Subsequent cooking to internal temperatures of 110° and 120°F resulted in reductions of 4.29 and 5.14 log CFU/g in *Salmonella* spp. Holding of blade-tenderized prime rib cooked to internal temperatures of 100° and 120°F and subsequently held in an oven at 140°F resulted in an increase of 2.36 log CFU/g or survival (<0.09 log CFU/g increase) in *Salmonella* spp. levels.

Significance: Reductions in *Salmonella* spp. were observed during preparation of rare prime rib. However, use of non-intact cuts of beef is not recommended for preparation of beef dishes that are cooked to low temperatures with long holding times.

P1-61 Thermal Inactivation of F-RNA Coliphages in Beef Slurries Determined by Culture and Quantitative Real-time RT-PCR

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Introduction: Most potential pathogenic enteric viruses are detected by molecular techniques, but the significance of the presence of viral nucleic acid is questionable when foods are subjected to thermal inactivation treatments. F-RNA coliphages may be deposited on meat along with other enteric organisms during carcass processing. F-RNA coliphages are possible surrogates/indicators for potential zoonotic enteric viruses. Furthermore, F-RNA coliphage MS2 has similar survival characteristics as human enteric viruses and can be rapidly cultured.

Purpose: Information on the heat resistance of MS2 and environmental F-RNA coliphages at temperatures relevant to foods is lacking. F-RNA coliphage was used as a culturable surrogate virus to examine relationships between potential infectivity and the detection of viral nucleic acid in foods in response to exposure to heat inactivation treatments.

Methods: MS2 was inoculated at 10^8 plaque forming units (PFU)/g in lean and 15% fat beef slurries in whirl-pack bags and immersed in a waterbath at temperatures ranging from 60 to 72°C for up to 30 minutes, in duplicate. The survival of 10 environmental F-RNA coliphage isolates were compared to MS2 in lean beef slurries at 66°C. Numbers of PFU and genome copies (gc) were determined at regular intervals. Gc of F-RNA coliphages and feline calicivirus (a sample process control) were determined by multiplex real time RT-PCR.

Results: The recovery of PFU or gc of MS2 was similar in lean and 15% fat slurries. MS2 remained viable after 30 min at 60 and 63°C. After 1 and 30 min in lean slurry, the reductions of MS2 were 1.1 and 2.6 log PFU or 1.1 and 1.4 log gc at 60°C and 2.9 and 7.0 log PFU or 1.9 and 2.6 log gc at 66°C, respectively. After 1 min at 72°C, the reduction was 5.9 log pfu and 2.9 log gc. After 1 min at 66°C, reductions for environmental F-RNA strains ranged from 2.6 to 3.8 log PFU, with an average of 3.2 log PFU.

Significance: MS2 is a representative model for environmental F-RNA coliphages in heat inactivation studies. The lack of a relationship between infectivity and detection of viral nucleic acid increased with increasing temperatures, an important consideration when molecular methods are used for the detection of viruses in heat-treated foods.

P1-62 *Salmonella* in Lymph Nodes of Cattle Presented for Harvest

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Introduction: Salmonella can invade and survive within host immune cells. Once internalized, these pathogens have the potential to disseminate throughout the lymphatic system and reside within lymph nodes. If so, because some lymph nodes are located within muscle and fat tissues, *Salmonella*-positive lymph nodes may potentially be incorporated into ground beef.

Purpose: The purpose of this study was to determine the prevalence of *Salmonella* in subiliac cattle lymph nodes obtained from cattle.

Methods: Lymph nodes were collected from the carcasses of feedlot cattle and dairy cows in commercial packing plants prior to being trimmed to remove all fat and dipped in boiling water for three seconds before pulverization in a Whirl-pak bag and enrichment with 80 milliliters of tryptic soy broth. Enrichments were subjected to immunomagnetic separation (IMS). IMS beads were transferred to Rappaport-Vasiliadis Broth, incubated and these secondary enrichments were then streaked onto brilliant green sulfa agar and XLD agar. Presumptive *Salmonella* isolates were confirmed by *invA* PCR and serotyped.

Results: Between September and November of 2010, the mean prevalence of *Salmonella* in cattle lymph nodes (n=1,039) was 8.7% (95% CI 0.93 – 16.6%). The majority of *Salmonella* were Montevideo and Anatum.

Significance: Salmonella was readily recovered from subiliac lymph nodes and consequently, fat trim containing lymph nodes may be a point-source for *Salmonella* entry into ground beef products. The public health consequence of these findings should be further investigated and research is needed to better understand the routes of infection and opportunities to reduce the risk of *Salmonella* in lymph nodes of healthy cattle.

P1-63 Prevalence of Shiga Toxin-producing *Escherichia coli* in Internal Organs of Cattle Distributed for Food in Japan

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Introduction: Outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) infection are caused mainly by the consumption of contaminated foods. The most probable source is livestock products, especially cattle, and epidemiological data suggest that consumption of cattle offal significantly contributes to human illnesses in Japan. The prevalence of STEC in beef carcass meat distributed in Japan has been examined and annually reported, however, the internal organs other than livers are not included.

Purpose: The aim of this study was to examine the prevalence of STEC in cattle internal organs distributed for food in Japan.

Methods: 229 retailed offal samples were screened by PCR for shiga toxin (*stx*) gene after enrichment. The *stx*-positive samples were further screened for $rfbE_{O157}$, wzy_{O26} and rfb_{O111} genes. Immunomagnetic separation was conducted to isolate these serotypes of STEC. The obtained isolates were genotyped for *stx1, stx2, uidA, eaeA* and *ehxA* genes.

Results: 39 out of 229 samples tested (17.0%) were *stx*-positive and among them, eight and three samples were revealed to be $rfbE_{O157}$- and wzy_{O26}-positive (20.5% and 7.7%), respectively. None of them was found to be rfb_{O111}-positive. STEC isolates were obtained from three $rfbE_{O157}$-positive samples of small intestine and each one of $rfbE_{O157}$-positive and wzy_{O26}-positive omasum sample. Although *stx* genotype differed isolate by isolate and a wzy_{O26}-positive isolate was *uidA*-negative, all the five isolates possessed both *eaeA* and *ehxA* genes. The prevalence, i.e., isolation rate in offal samples, was not different much from that in carcass meat reported. However, spike experiments revealed that the growth of STEC O157 was inhibited in offal samples during pre-enrichment procedure, indicating that the detection limit was higher in offal than in carcass meat samples.

Significance: This is the first report demonstrating the prevalence of STEC in cattle internal organs commercially distributed for food in Japan. The results obtained in this study could provide important information in estimating risk due to consumption of these organs and further to the prevention of STEC outbreaks caused by cattle materials.

P1-64 Soil Solarization Reduces *Escherichia coli* O157:H7 on Cattle Feedlot Pen Surfaces

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Introduction: Soils at the feedlot pen surface are a source for transmission of *Escherichia coli* O157:H7, and therefore, a target for control measures to reduce this pathogen in cattle. Soil solarization is a preplanting technique used in food and ornamental crop production, which utilizes solar energy to heat the soil and inactivate plant pathogens, nematodes, weeds and weed seeds.

Purpose: The objective of this study was to determine the ability of soil solarization to reduce *E. coli* O157:H7 from feedlot pen surface soils.

Methods: A feedlot pen was identified in which *E. coli* O157:H7 was both highly prevalent and evenly distributed in the surface soils. Forty 3.0×3.0-meter plots were randomly assigned such that 5 plots of each of the solarization times of 0, 1, 2, 3, 4, 6, 8 and 10 weeks were examined. Button temperature loggers were placed 5 to 7.5 cm below the surface in the center of each plot, and plots were covered with 3.6×3.6-meter sheets of clear 6 mil polyethylene. At each sampling, 5 soil samples were collected from each of the 10 plots (5 solarized and 5 unsolarized plots; n = 25). Total *E. coli* concentrations on CHROMagar ECC and *E. coli* O157:H7 presence by immunomagnetic separation and plating were determined for each soil sample.

Results: Initial percentages of *E. coli* O157:H7-positive samples in solarized and unsolarized plots were 80 and 84%, respectively, and did not differ ($P > 0.05$). *E. coli* O157:H7 was no longer detectable by 8 weeks of solarization, but was still detected in unsolarized soils at 10 weeks. The average initial concentration of *E. coli* in soils was 5.56 log CFU/g, and did not differ between treatments ($P > 0.05$). There was a rapid 2.0-log decrease of *E. coli* after 1 week of solarization, and >3.0-log reduction of *E. coli* by week 6 of solarization ($P < 0.001$). *E. coli* concentrations remained unchanged in unsolarized soils after 10 weeks ($P > 0.05$). Average daily maximum temperatures were 10 to 12 degrees higher for solarized soils compared to unsolarized soils, and reached temperatures as high as 55°C.

Significance: Because soil solarization effectively reduces *E. coli* O157:H7 from feedlot pen surface soils, it may be useful for reducing the transmission and persistence of this pathogen among cattle and the production environment. In addition, this technique may have application for remediation of *E. coli* O157:H7-contaminated soils used to grow food crops.

P1-65 Inhibition of *Clostridium perfringens* Spore Germination and Outgrowth in Reduced NaCl Roast Beef by Buffered Lemon Juice and Vinegar Product

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Introduction: Processed meat and poultry products that are improperly cooled or temperature abused have a greater potential to cause *Clostridium perfringens* illness. Consumer preference for meat products containing reduced sodium may present a greater risk of *C. perfringens* spore germination and outgrowth.

Purpose: Inhibition of *C. perfringens* spore germination and outgrowth during abusive cooling in reduced sodium roast beef by buffered lemon juice and vinegar concentrate (LV; MoStatin LV, WTI, Inc.) was evaluated.

Methods: Roast beef was prepared to contain NaCl (1, 1.5, or 2%, wt/wt), phosphate (0.3%) and various concentrations of LV (0, 2, or 2.5%). Roast beef treatments (5 g) were placed in a vacuum bag and inoculated with a three-strain cocktail of *C. perfringens* spores (final spore population of ca. 2.5 log CFU/g) and vacuum packaged. The product was heat treated and chilled exponentially from 54.4 to 4.4°C within 6.5, 9, 12, 15, 18 or 21 h.

Results: Cooling of roast beef (2.0% NaCl) within 6.5 and 9 h resulted in <1.0 log CFU/g increase in *C. perfringens* spore germination and outgrowth, whereas reducing the NaCl concentration (to 1.5 or 1.0%) resulted in >1.0 log CFU/g increase for cooling times 9 h (1.1 and 2.2 log CFU/g, respectively) and longer. Incorporation of LV minimized the *C. perfringens* spore germination and outgrowth to <1.0 log CFU/g, regardless of the NaCl concentration and cooling time.

Significance: Increased risk of *C. perfringens* spore germination and outgrowth during chilling roast beef can be expected with reduction in NaCl in roast beef formulations. However, *C. perfringens* spore germination and outgrowth can be minimized by incorporation of buffered lemon juice and vinegar concentrate in roast beef formulations.

P1-66 Control of *Clostridium perfringens* Germination and Outgrowth in Reduced-sodium Roast Beef during Abusive Cooling by Sodium Diacetate

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Introduction: Recent reports on health benefits of reducing sodium content in human diet have resulted in greater variety of reduced-sodium meat products. However, reducing the sodium chloride (NaCl) in meat products may allow germination and outgrowth of *Clostridium perfringens* spores during abusive cooling or holding.

Purpose: Control of *C. perfringens* spore germination and outgrowth by sodium diacetate (Bombal™) in roast beef with reduced sodium chloride during abusive cooling was evaluated.

Methods: Roast beef was formulated to contain NaCl (1.23%), reduced NaCl (0.86%) or NaCl-KCl (0.86%-0.37%); phosphate (0.3%) and Bombal™ (0, 0.35, or 0.50%). Aliquots (5 g) of the roast beef were placed in a vacuum bag, inoculated with a three-strain *Clostridium perfringens* spore cocktail to obtain ca. 2.5 log spores/g and vacuum packaged. The inoculated roast beef was heat treated for 20 min at 75°C and chilled exponentially from 54.4 to 4.4°C within 6.5, 9, 12, 15, 18, or 21 h.

Results: Reduction of sodium chloride or replacement with KCl did not affect *C. perfringens* spore germination and outgrowth (< 1.0 log CFU/g) within 6.5 h of cooling; where as higher populations (3.23 and 1.09 log CFU/g, respectively) were observed during 9 h cooling. Incorporation of Bombal™ inhibited germination and outgrowth of *C. perfringens* in roast beef dependent on the concentration and cooling time.

Significance: Incorporation of Bombal™ into reduced-sodium roast beef formulations can minimize the risk of *C. perfringens* spore germination and outgrowth to <1.0 log CFU/g during marginal temperature abuse (<9 h) during cooling.

P1-67 Dynamic Model to Predict *Salmonella* Growth in Fresh Pork during Distribution and Storage

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Introduction: Since storage temperature is changed during distribution and storage of fresh pork cutting, the mathematical model predicting bacterial populations at changing temperature is necessary.

Purpose: This study developed mathematical models to predict *Salmonella enterica* growth in fresh pork cutting under constant and dynamic storage temperature conditions.

Methods: Sterile fresh pork tenderloin slices (0.5 x 2.5 x 5 cm) were prepared by gamma-irradiation at 40 kGy. *Salmonella* (2 log CFU/cm^2) was inoculated into pork slices and they were stored at 4, 15, 25, and 35°C up to 240 h, depending on storage temperature. Bacterial populations of *Salmonella* were measured with tryptic soy agar (TSA) during storage. For calculation of kinetic parameters such as maximum growth rate (μmax, log CFU/cm^2/h), lag phase duration (h), low asymptote (N0), and upper asymptote (Nmax), cell counts recovered with TSA were fitted to the Baranyi model, and these parameters were further expressed for the development of the secondary models and dynamic model.

Results: Squared root of kinetic parameters derived from the Baranyi model were well-described storage temperature effect on behavior of *Salmonella* (R^2: 0.800-0.992). In addition, a dynamic model was also developed to predict growth of *Salmonella* at changing temperature, and various indices showed that model performance was acceptable for both temperature conditions (Bias factors: 1.009-1.011; Accuracy factors: 1.038-1.040; R^2: 0.963-0.977; standard error of estimate: 0.288-0.298).

Significance: These results suggest that the developed mathematical models could be useful in predicting *Salmonella* growth in fresh pork, which may result in improved food safety in the meat industry.

P1-68 Survival and Growth of *Campylobacter jejuni* and *Salmonella enterica* Typhimurium in Moisture-enhanced Pork during Vacuum Storage

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Introduction: Moisture-enhanced pork is a common non-intact meat product. Concerns about the microbiological safety of such non-intact meat products have been raised for many reasons. For example, bacteria translocation occurs from the exterior into the interior through moisture enhancement. People preparing dishes with these meats may regard them as intact products (only thoroughly cooking surface tissue).

Purpose: The present study was conducted to determine the effects of refrigeration, abuse temperature and moisture enhancement on the survival of *Campylobacter jejuni* and *Salmonella enterica* Typhimurium in moisture-enhanced pork during vacuum storage.

Methods: Pork loins were surface inoculated with either *Campylobacter jejuni* or *Salmonella* Typhimurium, and then subjected to a single pass through a needle injector moisture enhancement process with a target injection volume of 10% and 20%. The moisture-enhanced pork loins were sliced into 1 cm thick slices and vacuum packaged. Samples were collected, plated and the populations of survival organisms were analyzed periodically during storage at 4°C and 10°C.

Results: There was no significant effect of moisture-enhancement on the populations of *Campylobacter jejuni* and *Salmonella* Typhimurium in samples ($P > 0.05$). After 28 days, the populations of *Campylobacter jejuni* and *Salmonella* Typhimurium in samples were significantly lower ($P < 0.05$) than those of Day 0. Mean populations of *Campylobacter jejuni* and *Salmonella* Typhimurium in samples at Day 28 were 4.24, 4.78 $\log_{10}$CFU/g, respectively. No significant differences ($P > 0.05$) in *Campylobacter jejuni* counts were observed between samples at abuse temperature (10°C) and those at the refrigerated temperature (4°C). In contrast, the population size of *Salmonella* Typhimurium in samples at abuse temperature (10°C) was significantly ($P < 0.05$) higher than those at refrigerated temperature (4°C).

Significance: This study indicates that, vacuum packing under chilled conditions alone is not a substitute for safe handling and proper cooking. The event of temperature abuse during handling should be avoided.

P1-69 The Influence of Temperature, Relative Humidity, and Concentration of Sausage Solids on the Colonization of *Leuconostoc mesenteroides* on Polypropylene

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Introduction: Deficient sanitation practices and biofilm formation could lead to the persistence of spoilage microorganisms in food processing environments. *Leuconostoc* spp. is able to adhere and colonize inert surfaces such as polypropylene. Ecological factors including temperature, relative humidity and nutrient concentration could promote the microbial colonization and biofilm formation.

Purpose: To determine the effect of temperature, relative humidity and sausage solids concentration on the colonization of *Leuconostoc mesenteroides* in polypropylene spheres.

Methods: L. mesenteroides strains isolated from a sausage processing environment and a model of polypropylene spheres were used. Adhesion was promoted by immersing the spheres (30°C/4h) in a sausage suspension (0.5%w/v) containing 9 log CFU/ml of *L. mesenteroides*. The unattached cells were removed by wash with isotonic saline solution. Spheres containing attached cells were immersed in sterile sausage suspensions (1 and 5%w/v) during 20 min, then dried in laminar flow, and finally stored at 4°C and 22°C inside of containers equilibrated at 60, 86 and 97% of relative humidity (RH). Additionally, spheres containing attached cells were immersed in the sausage suspensions and designated as 100% of RH. Periodically, attached cells were removed and quantified in MRS agar. Growth data were fitted with DMfit program (www.combase.cc).

Results: Sausage solids concentration did not influence ($P < 0.05$) the growth rate of *L. mesenteroides* during its colonization on the polypropylene spheres. In contrast, the temperature and RH interaction showed a significant influence on that parameter. The growth rate values estimated for *Leuconostoc* colonization at 4°C were, 0.151 and 0.080 log CFU/h at 100 and 97% of RH, respectively. No growth was observed at 86 and 60% of RH at 4°C. At 22°C, the growth rate values were: 0.330, 0.415, 0.290 and 0.175 log CFU/h for 100, 97, 86 and 60% of HR, respectively. The maximum population reached during colonization at both temperatures ranged between 5 to 7 log CFU/cm^2.

Significance: The control of temperature and RH in the food processing environment can reduce *Leuconostoc* colonization on polypropylene surfaces.

P1-70 The Effects of Initial Contamination Levels, Biofilm and Fat on the Desiccation Survival of *Listeria monocytogenes* on Stainless Steel Surfaces

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Introduction: Listeria monocytogenes is a serious concern for the food industry, as the pathogen can persist for extended periods of time on food processing surfaces. Recently it was discovered that salt and biofilm formation increase survival during long-term desiccation. Research into environmental factors affecting survival will ultimately aid in preventing future outbreaks.

Purpose: To investigate the effects of food components (fats) and environmental conditions (initial contamination levels, biofilm formation) on *L. monocytogenes* desiccation survival on food grade stainless steel (SS).

Methods: L. monocytogenes 568 was pre-cultured (48 h, 15°C) and re-suspended in Tryptic Soy Broth (TSB), adjusted to three different inoculation levels (~107.5, 105.5, 103.5 CFU/cm^2) and spotted on SS coupons (0.25 cm^2). Coupons were desiccated (43% relative humidity (RH), 15°C) for 20 d and survivors were enumerated on BHI agar. The same desiccation experiment was repeated on biofilm cells (48 h, 100% RH, 15°C). To test the effect of fat, bacteria were also desiccated in TSB containing animal fat (20%, 60%) or canola oil (5%, 10%). The Weibull model (log(N) = log(N0) - (t/delta)p) was used to model the non-linear inactivation kinetics.

Results: Without biofilm, initial cell density had no effect on desiccation kinetics and the desiccation-resistant sub-population size remained proportional to initial levels. During biofilm formation, only the two higher inoculation levels reached the maximum cell density (~108.5 CFU/cm^2) which led to higher delta values (time to first log reduction) as compared to low initial level biofilm (2.52 ± 1.10, 4.98 ± 1.35 and 0.45 ± 0.33 d for high, medium, and low levels, respectively). Fat levels ≥ 20% significantly ($P < 0.05$) increased survival (delta; 4.24 ± 1.33, 1.37 ± 0.62, and 0.20 ± 0.02 d for 60, 20 and 0%).

Significance: The presence of organic surface material as in biofilm or fat, but not initial cell densities, significantly increase desiccation survival of *L. monocytogenes* under simulated food processing environmental conditions.

P1-71 Survival of *Salmonella* Species on Stainless Steel Exposed to Dry Heat

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Introduction: Limited data are available regarding *Salmonella* survival on non-porous surfaces in dry conditions. In the dry goods industry, *Salmonella* contamination is a serious concern, as this pathogen is known to survive in several dry foods such as flour, nuts and dried milk products. Common practice in many dry food manufacturing plants involves maintaining a dry environment for lengthy periods of several weeks followed by a wet cleaning and sanitation cycle. In situations where the introduction of moisture is detrimental, heat may facilitate removal of unwanted microorganisms.

Purpose: The purpose of this study was to investigate the ability of *Salmonella* to survive on stainless steel in dry and moist-heat conditions.

Methods: A mixture of *Salmonella* Typhimurium ATCC 13311, *Salmonella* Choleraesuis ATCC 10708, and *Salmonella* Enteritidis ATCC 13076 was inoculated onto stainless steel carriers and exposed to several different dry and moist-heat conditions. After the indicated exposure times the organisms were recovered and log reductions were calculated.

Results: In dry conditions, at 26°C after 7 days, there was a 4.98 log reduction from the initial inoculum. After 4 days at 55°C, a 3.54 log reduction was observed. At 80°C there was a 1.85 log reduction after 4 h. Over a 4 log reduction was achieved after 4 h at 90°C or after 2 h at 100°C. When coupons were soiled with whole milk, a > 4 log reduction was achieved after 45 min. When a 5% glucose solution was used the same reduction was achieved after 15 min. In the presence of steam (80–90°C) a > 4 log reduction was observed after 5 min with or without the whole milk or glucose soils.

Significance: Results of this study indicate dry heat can eliminate *Salmonella* from non-porous surfaces, such as stainless steel, but will require extended time periods and/or high temperatures.

P1-72 Chlorine Resistance of *Listeria monocytogenes* Cells Recovered from Biofilms Exposed to Chlorine

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Introduction: Microorganisms are able to attach, to grow on, and ultimately form biofilms on industrial equipment and food contact surfaces. Attached microorganisms can be more resistant to disinfectants compounds than planktonic cells. This could be attributed to the physical barrier that biofilm offers or due to cell's adaptation.

Purpose: To evaluate the resistance to chlorine of *L. monocytogenes* cells recovered from pre-exposed biofilms to the germicide.

Methods: Eight strains of *L. monocytogenes* were used. From each strain three types of cells were prepared: Planktonic cells grown in tryptic soy broth. Cells recovered from biofilms generated on stainless steel chips. Briefly, stainless steel chips were inoculated with 100 µl of spinach suspension (20%) containing ~1000 cells and incubated at 30°C and 95% relative humidity for up to 12 days; at the end of the storage cells were removed from the chips. Cells recovered from biofilms (formed as described before) that were treated with chlorine (50 ppm, 60 s) along its formation (after 4, 6, 8 and 10 days). Cells from treatments A, B, and C were exposed to chlorine solution (~3 ppm) for 200 min at 30°C. Survivors were determined periodically on tryptic soy agar and data were fitted by the DMFit Excel Add-In available through the ComBase web site (www.combase.cc).

Results: After the challenge with chlorine, the log reductions and death rates values for *L. monocytogenes* strains subjected to treatments A, B and C were 7.91 and 0.0504; 5.26 and 0.0252; and 3.73 log CFU/ml and 0.0185 log CFU/h, respectively. Cells recovered from biofilms with or without pre-exposure to chlorine survived up to 200 minutes during chlorine challenge, whereas planktonic cells were not recovered after 160 minutes.

Significance: L. monocytogenes cells immersed in biofilms exposed periodically to chlorine showed more resistance to the disinfectant than their counterparts in biofilms without germicide contact. Further research is needed to determine the way in which bacteria become resistant.

P1-73 Action of Peracetic Acid on *Staphylococcus aureus* on Suspension, Settled on Stainless Steel or on NYLON Surfaces: Influence of the Settling Time

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Introduction: Peracetic acid (PAA) is an important sanitizer agent used in the food industry. Stainless steel (SS) and NYLON are used in food processing equipment. *Staphylococcus aureus* is a pathogenic microorganism that could be found on food processing surfaces.

Purpose: The objective of this study was to evaluate the influence of settling time on the activity of PAA over *Staphylococcus aureus* cells settled on SS and NYLON surfaces.

Methods: The destruction kinetics (DK) promoted by a 40 mg.L-1 PAA sanitizer was studied using the suspension method of AOAC when cells were in suspension, and the swab method when cells were settled on SS and NYLON surfaces.

Results: The DK for cells settled on surfaces was influenced by the settling time (t). Calling the contact time between the settled cells and the sanitizer, and D the decimal reduction time, the following facts were observed: 1) D = 2.4 min when cells were in a suspension containing the sanitizer; 2) when sanitizer was applied on cells settled on SS; a) and $t \leq 6$ h and < 25 min, the DK can be represented by a zero order equation; b) when t = 0.5 h, D = 2.6 min if > 25 min; c) when t = 6 h, D = 2.7 min if > 25 min; d) when t = 16 h, D = 13.3 min if ≤ 25 min and D = 5.0 min if > 25 min; e) when t = 24 h, D = 14.0 min if ≤ 30 min and D = 5.9 min if > 30 min. 3) when sanitizer was applied on cells settled on NYLON and t = 0.5 h, D = 14.6 min.

Significance: These results show that sanitation of SS is easier done than NYLON. Therefore, more attention should be taken when this type of polymer is used in food industries.

P1-74 Effect of an Ultrasound-sanitizer Combined Treatment in the Detachment of Bacteria on Food Contact Surfaces

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Introduction: Cross-contamination of fruits and vegetables can readily occur through contaminated surfaces, thus there is a need to develop methods to inactivate microorganisms on surfaces of various materials. The aim of this study was to develop combined methods such as using ultrasound and chemical sanitizers to detach *Escherichia coli* and *Staphylococcus aureus* on food contact surfaces as a function of concentration and exposure time.

Purpose: This study investigated the synergistic effect of ultrasound on chlorine disinfectant treatment for the reduction of *Escherichia coli* ATCC 10536 and *Staphylococcus aureus* ATCC 6538 on stainless steel surfaces.

Methods: E. coli ATCC 10536 and *S. aureus* ATCC 6538 were inoculated on stainless steel (SUS) chips and dried on a clean bench for 1 hr. These chips were treated for various exposure times (0-120 min) with 37 kHz and 1200 W of ultrasound and various concentrations (0-200 ppm) of chlorine. Single treatments of ultrasound and chlorine as well as their combined treatment were evaluated for the detachment of fixed bacteria.

Results: The single treatment of ultrasound for 120 min reduced 1.54 and 1.34 log CFU/coupon of *E. coli* and S. *aureus* on the SUS chips, respectively. And the single treatment of chlorine at 200 ppm for 5 min reduced 1.49 and 3.69 log CFU/coupon of *E. coli* and *S. aureus*, respectively. However, the combined treatment of ultrasound (37 kHz and 1200W) and chlorine (200 ppm) reduced 2.08 and 4.16 log CFU/coupon of *E. coli* and *S. aureus*, respectively. The additional reduction levels by the combined treatment were 0.54-0.59 and 0.47-2.82 log CFU/coupon of *E. coli* and *S. aureus*, respectively, compared to the single treatments. Although the combine ultrasound/chlorine treatment did not show a synergistic effect for reduction, it showed additional reductions with decreased exposure time to ultrasound and sanitizer concentration than the single treatments against foodborne pathogens.

Significance: Combined ultrasound and chlorine treatments can be used to increase detachment efficacy with additional reductions of bacteria and decreased treatment time and chlorine concentration.

P1-75 Synergistic Effects of Combined Disinfecting Treatments Using Sanitizers and UV to Reduce Levels of *Bacillus cereus* in Oyster Mushrooms

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Introduction: Reports of food poisoning outbreaks due to vegetables and fruits have increased. Therefore, various combined techniques are being popularly applied to the fresh food industry to reduce bacterial foodborne pathogens. In our study, both chemical disinfectant and UV irradiation are used to reduce *Bacillus cereus* in oyster mushrooms.

Purpose: The purpose of this study was to determine whether combined processing would produce synergistic effects in the disinfection of food products during food processing compared to single processing.

Methods: The oyster mushroom samples were purchased from a Korean local market. The reduction of *Bacillus cereus* F4810/72 by various concentrations of disinfectants such as ethanol (10, 30, 40, 50%), hydrogen peroxide (100, 500, 1000, 2000ppm), sodium hypochlorite (10, 50, 100, 200ppm) were tested with various exposure doses (6, 96, 216, 360 and 506 mWs/cm^2) of UV lamp (2,537Å).

Results: Three of the combined treatments of ethanol/UV, hydrogen peroxide/UV and sodium hypochlorite/UV exhibited a greater reduction and synergistic benefits. Reductions in the populations of *B. cereus* on the oyster mushroom slices were between 0.21 – 1.74 $\log_{10}$ CFU/g depending on the UV irradiation dose. The reduction values for *B. cereus* were between 0.16 – 3.02 (ethanol), 0.32 – 1.10 (hydrogen peroxide), 0.85 –1.75 (sodium hypochlorite) $\log_{10}$ CFU/g depending on the disinfectants concentration, compared to the control. The synergy values of combined ethanol/UV, hydrogen peroxide/UV and sodium hypochlorite/UV treatment for *B. cereus* were 0.14 –1.26 $\log_{10}$, 0.05 – 0.76 $\log_{10}$ and 0.09 – 0.9 $\log_{10}$ CFU/g, respectively. The result of this study means that a significant synergistic benefit results from combined sanitizer/UV treatment to reduce foodborne pathogens on oyster mushrooms.

Significance: Combined treatments of disinfectants and UV irradiation produce a synergistic reduction of *B. cereus* in mushrooms.

P1-76 Synergistic Effects of Ethanol and UV Radiation to Reduce Levels of Selected Foodborne Pathogenic Bacteria

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Introduction: In recent years, there have been an increasing number of outbreaks of food poisonings linked to the consumption of various foods in Korea. Various combined techniques are being popularly applied to the food industry to reduce the contamination level of foodborne pathogenic bacteria.

Purpose: The purpose of this study was to determine whether combined processing would produce synergistic effects in the disinfection of food products during food processing, compared to single processing. This study investigated the synergistic effect of UV irradiation and ethanol disinfectant treatments for reduction of *Bacillus cereus* F4810/72, *Cronobacter sakazakii* KCTC 2949, *Staphylococcus aureus* ATCC 35556, *Escherichia coli* ATCC 10536, and *Salmonella enterica* Typhimurium NO/NA in vitro.

Methods: The reduction of five foodborne pathogens by various concentrations of disinfectants such as 10, 30, 40, and 50% ethanol were tested with various exposure doses (6, 96, 216, 360 and 506 mWs/cm^2) of UV lamp (2,537Å).

Results: Reductions in the populations of *B. cereus, C. sakazakii, S. aureus, S.* Typhimurium, and *E. coli* in vitro were between 0.42-2.12, 0.43-1.64, 0.60-1.02, 0.41-2.48, and 1.08-2.32 $\log_{10}$ CFU/mL, respectively, depending on the UV radiation dose, compared to no treatment. The effects of 10, 30, 40, and 50% ethanol treatment alone on the five foodborne pathogenic bacteria in vitro were between 0.48-2.81, 0.73-3.81, 0.12-1.97, 0.13-2.01, and 0.05-2.91 $\log_{10}$ CFU/mL, respectively. The combined treatments of ethanol/UV exhibited a greater reduction and synergistic benefits. The synergy values of *B. cereus, C. sakazakii, S. aureus, S. enterica* Typhimurium NO/NA and *E. coli* were 0.40-1.52 $\log_{10}$ CFU/ml, 0.52-1.74 $\log_{10}$ CFU/ml, 0.20-2.32 $\log_{10}$ CFU/ml, 0.07-1.14 $\log_{10}$ CFU/ml and 0.02-1.75 $\log_{10}$ CFU/ml, respectively. The results of this study suggest that a significant synergistic benefit results from combined ethanol/UV processing against foodborne pathogens in vitro.

Significance: The combined ethanol and UV radiation treatments proved increased disinfection efficiency and synergistic effects in whole tests.

P1-77 Comparisons between Low-pressure Foam Cleaning and Conventional Cleaning to Remove Selected Bacterial Pathogens from Surfaces Associated with Convenience Food

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Introduction: With increased productivity, convenience foods, as well as other long shelf life processed foods, are affected by problems during cleaning and sanitation. The microbial status of eight convenience food manufacturing plants was determined by sampling stainless steel food contact surfaces after cleaning and sanitation at the end of a day's shift. 50% of the plants used conventional cleaning methods for cleaning and sanitation and 50% used the Low Pressure Foam (LPF) method.

Purpose: The purpose of this study was to develop data on the bacterial risks associated with contemporary and commercial cleaning methods used in the manufacturing of convenience foods in the Gauteng Province, South Africa. The objectives were to compare and evaluate the cleaning methods used for food contact surfaces, identify whether the selected organisms were present and to relate the bacterial count to the legal standard.

Methods: A total of 477 microbiological samples were taken from cleaned and sanitized convenience food contact surfaces on the basis of one manufacturing plant per day. A total of 205 samples were taken for Total Plate Count (TPC) tests, 79 microbiological samples to determine the presence of *Escherichia coli*, 27 microbiological samples to test for *Salmonella* spp. and *Staphylococcus aureus* each and 139 samples to test for the presence of *Listeria* spp. The samples were collected according to SABS method 763: swab technique by the researcher and trained research assistants and were analyzed on the same day according to accredited laboratory methods and techniques.

Results: Results revealed that 59% of the total areas sampled for TPC failed to comply with the legal requirements of the South African Health Act (< 100 CFU/cm^2). *Staphylococcus aureus* and *Salmonella* were not detected, but *Listeria* was detected in 23%, and *Escherichia coli* in 1.3% of the samples. A statistically significant difference ($P \leq 0.001$) was found in the TPC-means between both the cleaning methods and the eight manufacturing plants. No significant statistical difference was found between the presence of *Listeria* spp. and both the cleaning processes or the eight manufacturing plants. The LPF-method proved throughout to be the better cleaning option for reducing TPC-counts.

Significance: This study has highlighted the fact that pathogens remain viable on dry stainless steel surfaces after cleaning and sanitation and present a contamination hazard for considerable periods of time, depending on the contamination levels and type of pathogen. The results of this study emphasize the correct application of chemicals as well as intensive training for production workers in the convenience food industry.

P1-78 Inactivation Kinetics of *Escherichia coli* O157:H7 on Hard Surfaces by Use of a Bacteriophage Mixture

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Introduction: Food processing equipment, surfaces, and facilities can be contaminated by foodborne pathogens causing serious problems. Phages are obligate parasites of bacteria capable of killing specific species and offer a natural method to control contamination of foods.

Purpose: The objective of this study was to determine the effect of a previously characterized collection of bacteriophages, BEC8, on the inactivation kinetics of a mixture of EHEC O157:H7 strains applied onto surfaces of materials commonly found in food processing plants.

Methods: Chips made of sterile stainless steel (SS), ceramic tile (CT), and high density polyethylene (HDPE) were spot inoculated with 10^4 CFU/chip of a mixture of EHEC O157:H7 strains EK27, ATCC 43895, and 472 and dried or allowed to remain in liquid form. BEC8 (10^6 PFU/chip) was applied onto the inoculated surfaces to obtain a ratio of 100 PFU/CFU (multiplicity of infection) and incubated at 12, 23, 30 and 37°C. EHEC survival was determined using standard plate count on tryptic soy agar and rates of phage inactivation following first order kinetics and D- and Z-values were calculated.

Results: D-values on SS (R^2 values > 0.83) for liquid cells were 4.8, 4.6, 23.0, and 42.9 min and for dry cells 5.9, 8.8, 30.0, and 46.0 min; on CT (R^2 values > 0.71) for liquid cells were 3.9, 3.8, 21.3, and 32.6 min and for dry cells 5.1, 6.6, 29.3, and 46.7 min; on HDPE (R^2 values > 0.70) for liquid cells were 4.8, 5.2, 24.9, and 39.1 min and for dry cells 7.1, 7.0, 32.1, and 46.5 min at 37, 30, 23, and 12°C, respectively. Z-values for SS, CT, and HDPE for liquid cells were 23.2, 23.7, 24.5°C (R^2 values > 0.84) and for dry cells 26.1, 23.7, 26.7°C (R^2 values > 0.84), respectively.

Significance: These results indicated that bacteriophage cocktails can be used as an effective antimicrobial against EHEC O157:H7 on hard surfaces.

P1-79 Efficacy of Chemical Sanitizers on Foodborne Pathogens Contaminated on the Surface of Stainless Steel as Different Attached Form

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Introduction: Pathogens contaminated on the surface of utensils could contribute to the occurrence of outbreaks due to cross-contamination on foods during the food preparation process. It is difficult to control pathogens by sanitizing methods when they form a biofilm.

Purpose: The purpose of this study is to investigate biofilm formation of pathogens on the surface of stainless steel and to evaluate the efficacy of chemical sanitizers on inhibiting different types of pathogenic biofilm on the surface.

Methods: Stainless steel coupons inoculated with *E. coli* O157:H7 were stored in tryptic soy broth (TSB) at 25°C for 7 days or at 25°C and RH 100% for 21 days to investigate the formation of a biofilm. Chemical sanitizers (chlorine-based and alcohol-based commercial sanitizers) were used to treat different types of biofilm (attached form, biofilm formed in TSB broth, and biofilm formed at RH 100%) of *E. coli* O157:H7 and *S. aureus* on the surface of stainless steel.

Results: Levels of pathogens on the surface of stainless steel stored in TSB at 25°C for 7 days or at RH 100% and 25°C for 7 days were significantly increased. Increased levels after 1 day were ca. 3 log CFU/coupon, and these levels were maintained for 7 days, indicating pathogens formed biofilms on the surface of stainless steel. From the experiment comparing sanitizing methods, treatments with alcohol sanitizer were very effective on inactivating pathogenic biofilm on the surface of stainless steel. Reduction levels of alcohol sanitizer ranged from 1.91 to 4.77 log and from 4.35 to 5.35 log CFU/coupon in *E. coli* O157:H7 and *S. aureus*, respectively.

Significance: Alcohol-based sanitizers could potentially be used as a way to control microbial contamination on the surface of utensils, cooking equipment and other related environments regardless of their attached form.

P1-80 Comparison of Cleaning Methods for Reduction of Attached Microorganisms from Various Kitchen Cutting Boards

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Introduction: E. coli O157:H7 infection has been implicated in a number of outbreaks of illness linked to the consumption of foods. Kitchen cutting boards are important tools to be used for cooking; however, cross-contamination may occur between food and cutting boards.

Purpose: This study was conducted to investigate levels of cross-contamination of *E. coli* O157:H7 in ground beef to cutting boards made of different materials, and the effects of cleaning methods for reducing *E. coli* O157:H7 on various types of cutting boards.

Methods: Four types of commercial kitchen cutting boards (wood, polyethylene, polypropylene and acryl) were selected and contaminated with 50 g of ground beef inoculated *E. coli* O157:H7. Levels of cross-contaminated *E. coli* O157:H7 were evaluated. For testing cleaning methods, four different treatments (washing with a dry towel, wet towel, alcohol paper, and chlorine paper) were used to clean contaminated cutting boards.

Results: Initial populations of pathogens were 6.09 log CFU/g in ground beef. After 1 h placement of inoculated ground beef on cutting board, levels of cross-contaminated *E. coli* O157:H7 on the surface of cutting board of wood and polyethylene were 2.45 and 2.41 log CFU/100 cm^2, respectively, and there was no significant difference on levels of cross-contaminated *E. coli* O157:H7 depending materials except for normal polyethylene surface. From experiment for cleaning methods, efficacy of cleaning methods in removing *E. coli* O157: H7 was as follows: alcohol > chlorine > wet > dry paper towel. Therefore, cleaning with alcohol sanitizer seemed to be the most effective cleaning method to remove *E. coli* O157:H7 on kitchen cutting boards.

Significance: These results could be used to develop kitchen safety guidelines for preventing foodborne outbreaks.

P1-81 Survival of Pathogenic Bacteria on the Surface of Stainless Steel at Various Levels of Relative Humidity Depending on Different Attached Form

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Introduction: Various bacteria, including food spoilage bacteria and pathogens, could form biofilms on various food processing surfaces, potentially leading to food contamination.

Purpose: The purpose of this study was to evaluate survival of foodborne pathogens on the surface of stainless steel at different attached form stored at different levels of relative humidity.

Methods: Escherichia coli O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Cronobactor sakazakii* were inoculated on the surface of stainless steel as different attached forms (attached form, biofilm formed in TSB broth, and biofilm formed at RH 100%) and stored at different levels of relative humidity (RH 23%, 43%, 68%, 85%, and 100%) at room temperature for 5 days.

Results: When stainless steel coupons were stored at 25°C for 5 days, levels of pathogens on the surface of stainless steel were significantly reduced after storage at RH 23%, 43%, 68%, and 85% for 5 days except for RH 100%. When they formed biofilm on the surface of stainless steel in TSB, survival of pathogens showed similar results with their attached form. However, levels of *S. aureus* and *C. sakazakii* biofilm on the surface of stainless steel were slowly reduced at RH 23%, 43%, 68%, and 85% for 5 days compared with other pathogens. Biofilm formed at RH 100% was the most resistant among types of attached form and highly survived at all tested RH values.

Significance: From these results, survival of pathogens contaminated on the surface of food processing environment such as stainless steel could be different at various RH values depending on types of their attachment forms.

P1-82 Effect of Alternative Household Sanitizing Formulations to Inactivate Foodborne Pathogens on Food Contact Surfaces

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Introduction: Current trends indicate that American consumers are increasingly selecting products that they believe to be environmentally friendly or "natural." In the kitchen, this trend has been expressed through greater desire for using alternative or "green" sanitizers instead of common chemical sanitizers.

Purpose: The primary purpose of this work was to evaluate the effectiveness of one suggested alternative, tea tree oil, as a food contact surface sanitizer.

Methods: Three foodborne bacterial pathogens (*Listeria monocytogenes* N3-031, *Escherichia coli* O157:H7 E009, and *Salmonella* Typhimurium ATCC 14028) were applied separately onto three different food contact surfaces (high density polyethylene, glass, and Formica® laminate). Tea tree oil (TTO), borax, and vinegar (5% acetic acid) were applied individually as well as in combination for a total of seven treatment solutions. In addition, sterile reverse osmosis (RO) water and no applied treatment were used as controls. Treatments were tested using an adaptation of the Environmental Protection Agency DIS/TSS-10 test method whereby each contaminated surface was treated with 100 µl of test solution and held for 1 min followed by submersion in neutralizing buffer and microbiological plating. Samples (0.1 ml) were plated onto TSA and incubated at 35°C for 48 h prior to colony enumeration.

Results: TTO produced reductions between four and five logs for *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium and was not statistically different from the vinegar treatment ($P > 0.05$). All combination recipes, including the borax treatment, failed to produce reductions in microbial populations at levels considered to be appropriate for food contact surface sanitizers. Surface type did not play a significant role in the effectiveness of the treatment ($P > 0.05$).

Significance: Although TTO and vinegar did reduce pathogen populations on surfaces, reductions were not sufficient enough to be considered an equally effective alternative to common household sanitizers.

P1-83 A Comparison of Cleaning Fabrics for Bacterial Removal from Food Contact Surfaces

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Introduction: Food contact surfaces are highly contaminated with microorganisms and are great sources for transmission of foodborne pathogens. It is important to eliminate bacteria using appropriate sanitizing tools to minimize cross-contamination during food preparation and/or consumption and to reduce the risk of foodborne diseases.

Purpose: The objective of this study was to compare the removal efficiency of bacteria on food contact surfaces by different cleaning cloths.

Methods: Commercially available sponge-like cloths, scrubbing cloths, non-woven fabric and terrycloth were used. Formica was inoculated with *Listeria monocytogenes* in ready-to-eat turkey slurry and the surface was cleaned with different fabrics. The remaining bacteria on the food contact surfaces and bacteria immersed in each fabric were enumerated.

Results: Overall 2.3 to 4.2-log reductions were observed on Formica compared to positive control. Scrubbing cloths are designed to physically remove dirt attached to surfaces; however, they are not ideal to remove bacteria. Remaining bacterial levels after using scrubbing cloths were 2.83 ± 0.46 CFU/cm^2, and ATP bioluminescence assay results yielded 503.0 RLU. Terrycloths can retain considerable quantities of water due to their large surface areas, however the fabric exhibited low efficiency with a remaining bacterial level of 2.73 ± 0.52 CFU/cm^2 recovered, and an ATP assay response of 442.1 RLU. Non-woven cloths are commonly used for wipes but are considerably porous with the ability to absorb bacteria from surfaces. These cloths yielded recoverable levels of 2.46 ± 0.51 CFU/cm^2 and ATP assay responses of 443.9 RLU. Sponge cloths consisting of cotton and cellulose contain pores that would allow bacteria to penetrate into the cloth material and yielded recoverable levels of 1.07 ± 0.76 CFU/cm^2 and 208.0 RLU for the ATP test. All cleaning fabrics were treated with microwave for 1.5 min at 600W and no bacteria remained in all fabrics.

Significance: Overall, sponge-like cloth appears to have potential application for effective removal of bacteria from food contact surfaces.

P1-84 Efficacy of Sodium Hypochlorite and Ethanol against Norovirus-like Particles

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Introduction: A wide variety of effective disinfectants and sanitizers against the human norovirus (NoV), which causes food and waterborne outbreaks of gastroenteritis worldwide, are needed. For initial virucidal effectiveness tests of disinfectants against NoV, surrogate viruses such as the feline calicivirus (FCV) or murine norovirus (MNV) have been used because of the lack of a practicable cell assay system. Recently, we developed a new method of evaluating the efficacy of disinfectants against NoV virus-like particles (VLPs), whose three-dimensional protein structure and immunological properties are known.

Purpose: The purpose of this study was to determine the mechanism of inactivation of NoV by versatile disinfectants using our new method of evaluating efficacy using NoV VLPs as a surrogate for NoV.

Methods: Sodium hypochlorite and ethanol were mixed with an NoV VLPs stock solution. After exposure for 30, 60, and 180 s at room temperature, the disinfectants were neutralized with 100 mM $Na_2S_2O_3$ or diluted with distilled water. The samples were immediately analyzed by transmission electron microscopy (TEM), and the number of NoV VLPs with normal morphology was calculated. The morphological changes of the NoV VLPs protein were examined by SDS-PAGE.

Results: After the treatment with sodium hypochlorite at >500 ppm, >30 s, which were the concentrations and contact times effective against FCV and MNV as a surrogate viruses for NoV, NoV VLPs were not observed by TEM and the band corresponding to the major capsid protein (VP1) of NoV, the building block of NoV VLPs, was not detected by SDS-PAGE. After the treatment with ethanol at 50–70%, > 30 s, which were the concentration and contact time effective against MNV, few particles with normal morphology were observed by TEM but no change in the molecular weight of the primary structure of VP1 was observed.

Significance: The findings suggest that sodium hypochlorite and ethanol act differently on the particles. This method using NoV VLPs as a surrogate for NoV may contribute to improving our understanding of the inactivation mechanism of NoV by versatile disinfectants and to screening for other effective disinfectants and sanitizers in the future.

P1-85 Inactivation through Chlorine Treatment and Development of Predictive Models against Feline Calicivirus and Murine Norovirus on Food Contact Surfaces

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Introduction: Diverse disinfectants are used to inactivate viruses as well as bacteria. In particular, chlorine is a popular sanitizer to prevent cross-contamination of norovirus (NoV) on stainless steel surfaces used for food preparation and manufacture.

Purpose: This study was conducted to clarify the concentration and the optimal treatment time of chlorine for reducing feline calicivirus (FCV) and murine norovirus (MNV) on stainless steel surfaces, and to develop predictive inactivation models using response surface methodology (RSM).

Methods: The reduction levels of FCV and MNV on stainless steel surfaces after chlorine treatment with various concentrations (0–5,000 ppm) and treatment times (0–5 min) were investigated. Polynomial equations were derived to predict the performance of various chlorine concentrations and treatment times for reductions of FCV and MNV.

Results: The reduction values of FCV and MNV on stainless steel surfaces after 5,000 ppm of chlorine treatment were 5.20 TCID50/coupon and 5.20 TCID50/coupon, respectively. The reduction values by chlorine treatment against FCV and MNV were similar and were effective for viral inactivation. The polynomial equations predicting the inactivation of FCV and MNV were as follows: FCV (log TCID50/coupon) = -0.37135+0.836242x1 + 1.14357E-003x2 + 1.46800E-004x1x2 -0.11425x12 - 1.77048E-007 x22 (x1 ; time and x2 ; concentration); and MNV (log TCID50/coupon) = +0.047126 + 0.080690x1 + 1.10156E-003x2 + 8.960008E-005x1x2 -0.090979x12 - 1.62979E-007 x22 (x1 ; time and x2 ; concentration). The predictive inactivation models by RSM fit well (R^2 = 0.9532 and 0.9944, respectively) and were expressed as adequate models by Prob > F-value (P < 0.0001).

Significance: Chlorine treatment of stainless steel surfaces offers significant reductions of FCV and MNV, and predictive inactivation models may be applied for food preparation and manufacture.

P1-86 Novel Method of Evaluation of Disinfectants against Norovirus Virus-like Particles as a Surrogate for Human Norovirus

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Introduction: Human norovirus (NoV) is a major cause of food and waterborne outbreaks of gastroenteritis. Sanitation is one of the most important countermeasures to prevent the spread of NoV contaminations and infections. The development of a wide variety of effective disinfectants and sanitizers against NoV is urgently needed. However, there is no generally accepted method of evaluating NoV inactivation since NoV has no cell culture system. Surrogate viruses such as feline calicivirus (FCV) or murine norovirus (MNV) are commonly used for initial virucidal effectiveness tests.

Purpose: The purpose of this study was to develop a new evaluation method for NoV disinfection using NoV virus-like particles (VLPs) as a surrogate, whose three-dimensional protein structure and immunological properties are well known.

Methods: NoV VLPs were produced in a baculovirus expression system using *Spodoptera frugiperda* ovarian cells. The NoV VLPs were treated with different disinfectants, including sodium hypochlorite, alkali (Na_2CO_3, K_2CO_3, NaOH, and KOH), and EDTA. After treatments for 15 - 180 s at room temperature, the disinfectants were neutralized with 100 mM $Na_2S_2O_3$ or diluted with HEPES buffer. The treated NoV VLPs were analyzed by transmission electron microscopy and the numbers of particles with normal morphology were tallied.

Results: After the treatment with sodium hypochlorite (between 200 ppm - 500 ppm free chlorine) for 180 s, the numbers of normal morphology NoV VLPs decreased with concentration and time, whereas that of control, NoV VLPs treated with deactivated sodium hypochlorite, did not. After the treatment with > 500 ppm of free chlorine for > 30 s, NoV VLPs were not observed. After the treatments with alkaline disinfectants (> 50 mM) and EDTA (> 50 mM) for 180 s or more, NoV VLPs were degraded completely. These results suggested that the alkaline disinfectants and EDTA could be used as a NoV disinfection.

Significance: Our method of evaluating NoV inactivation offers a novel way to screen NoV disinfectants.

P1-87 Survival of *Salmonella, Escherichia coli* O157:H7*, Microsporidia* and *Cryptosporidium parvum* at Various Temperatures and Chemical Treatments

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Introduction: Foodborne illnesses have been associated with consumption of fresh produce and with minimally processed foods. Berries, green vegetables such as spinach, cilantro and green onions are frequently consumed raw, and if contaminated, could be responsible for illnesses in humans. Whether the current methodologies used to preserve and wash are sufficient to inactivate these pathogens has not been determined.

Purpose: The objectives of our study were to determine if bacterial pathogens, microsporidia and *Cryptosporidium* could survive the process of blanching greens prior to freezing and freezing fruits using sugar or syrup. In addition, we wanted to determine the effectiveness of chemical washes and systems containing ozone/silver/ultrasonic waves when cilantro leaves were experimentally inoculated with all four pathogens.

Methods: Six produce items (raspberries, strawberries, cilantro, spinach, basil and parsley) were inoculated with a 5-isolate cocktail of *E. coli* O157:H7, 5-isolate cocktail of *Salmonella* spp, *Encephalitozoon intestinalis* spores, and *Cryptosporidium parvum* oocysts. Freezing (whole or sliced fruit) and blanching (spinach leaves) at various periods of time were examined. In addition, chemical sanitizers were examined after 1 and 5 minute treatment. Bacteria survival was determined using selective microbiological agar media. Microsporidia and *C. parvum* infectivity was determined using the RK-13 and the HCT-8 cell lines, respectively.

Results: No effect on viability of the four foodborne pathogens was observed in the present study when the sanitizers were used to wash vegetables. Freezing did not kill the bacterial pathogens. A reduction of less than 2 logs was observed in microsporidia and less than 3 logs in *Cryptosporidium*.

Significance: Freezing did not inactivate both bacterial pathogens but reduced viability of microsporidia and *Cryptosporidium*, and the sanitizers used in the present study did not inactivate parasites or microsporidia but reduced bacteria viability. There is a need to identify disinfection and sanitation strategies that can control bacterial, as well as, parasitic contaminants which could be present in fresh produce and fruits and in minimally processed food items.

P1-88 Microbiological Analysis of Surfaces and Workers' Hands in Child Care Facilities in North and South Carolina

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Introduction: About 60% of U.S. children, age 5 and younger, spend time in child care. Such environments increase risk of enteric disease, including foodborne illness.

Purpose: To perform microbiological analysis on environmental samples obtained from child care facilities in the southeastern U.S.

Methods: Twenty-nine child care facilities were visited, 23 (79.3%) centers, 5 (17.2%) homes and 1 (3.4%) pre-school. For each visit, data collected included questionnaires, audits, observations and environmental samples. The latter (8-12 samples per facility) were collected from common surfaces (faucets, toys, refrigerators, diaper changing areas) and provider and food worker hands. These were analyzed for total aerobic bacteria (APC), coliforms, and generic *Escherichia coli*, as well as for *Shigella*, *Salmonella*, *E. coli* O157:H7 and *Campylobacter jejuni*.

Results: A total of 248 environmental samples were collected: 56 hand rinsates, 61 regular (flat) surfaces (FlS; 100 cm^2), and 131 irregular surfaces (IrS; e.g., faucets, toys). For hands, median APC and coliform counts were 4.5 and 1.7 log_{10} CFU/hand, respectively. For common surfaces, median APC was 2.3 (FlS) and 2.7 (IrS) log_{10} CFU/surface sampled, with median coliform counts 1.3 log_{10} CFU/surface. Coliforms were detected in 39 of 248 (15.7%)

samples with counts ranging from 1 to > 4.3 $\log_{10}$ CFU per sample. Generic *E. coli* was below detection limits (< 1 $\log_{10}$ CFU per sample) for all samples. No samples were positive for pathogens.

Significance: Absence of pathogens and generic *E. coli* suggests child care facilities managed fecal contamination well. When elevated coliform and APC counts did occur (defined as > 5.3 $\log_{10}$ CFU per sample),they were mostly associated with hands. Further analyses of samples for evidence of human norovirus and rotavirus are underway. These results, in conjunction with observational data, will be used to identify risk factors for enteric disease transmission and contamination of food in child care facilities.

P1-89 Assessment of the Level of Bacterial Contamination of Eating Utensils in Military Dining Halls in Turkey

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Introduction: Foodborne illness outbreaks associated with contaminated food preparation utensils have been widely reported in the literature. One source of contamination that has not been well studied is eating utensils. Eating utensils are exposed to a large number of people through everyday use and so could easily become contaminated and be a source for pathogenic bacteria if not properly cleaned and sanitized.

Purpose: To determine the level of bacterial contamination of eating utensils used in four military dining halls in Turkey.

Methods: Seventy (70) samples were collected from four dining halls located on one military post in Turkey. Swabs with an average area of 25 cm2 were taken with cotton swabs from forks (N=18), spoons (N=18), plates (N=18), and salt shakers (N=16) after the eating utensils were cleaned and while in storage. Samples were analyzed for generic *Escherichia coli* using Violet Red Bile Agar (VRBA). The swabs were also cultured onto 5% sheep blood agar plates and *S. aureus* isolates were identified by colony morphology, Gram staining, catalase, tube-coagulase, DNase, and mannitol tests. Final identification was performed with the Phoenix, an automated bacteriology system.

Results: Sixteen samples (22.8%) were positive for generic *E. coli* and 34 samples (48.5%) for *S. aureus*. Specifically, evidence of generic *E. coli* was found in 45%, 40%, 14%, and 10% of spoons forks, salt shakers, and plates, respectively. *S. aureus* was isolated from 90%, 86%, 60%, and 55% of plates, salt shakers, forks, and spoons, respectively.

Significance: Eating utensils can be a source of pathogenic microorganisms if not properly cleaned and sanitized. Methods for cleaning and sanitizing used in military dining halls must be reviewed to be certain that appropriate sanitization is being achieved.

P1-90 New Natural Food Preservative for RTE (Ready-to-Eat) Vegetables

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Introduction: Galla Chinensis (GAC) has been used traditionally to treat inflammation, dysentery, prolonged coughing, toxicosis and so on in Asian countries. It has been reported that GAC has antimicrobial and antioxidant activity. Therefore, GAC could be an appropriate candidate for a natural food preservative for RTE (Ready-to-Eat) vegetables. Recently, RTE vegetables became popular foods in Korea. This study dealt with GAC extracts as a natural food preservative to promote functionality and safety.

Purpose: Currently more natural food preservatives replace traditional sanitizing agents, such as chlorine solutions. The purpose of this study is to develop new natural food preservatives made of GAC extracts, and to evaluate their efficiencies in terms of functionality and safety.

Methods: GAC was grounded and kept at 4°C until needed. 50g of GAC was extracted with 5 different kinds of ethanol solutions (0, 30, 50, 70, 100% drinkable ethanol) for 3 h at 80°C. A disc diffusion method and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay were used to evaluate antimicrobial and antioxidant activities, respectively. In addition, the total phenolic compounds in GAC was measured. For measuring the applicability of the natural preservative for RTE vegetables, changes of weight, color, pH, and amount of microorganism were measured. Furthermore, sensory evaluation for the preservative was conducted.

Results: GAC extracts had stronger antimicrobial activities against *S. aureus*, *E. coli*, and *B. cereus* than commonly used food preservatives such as sodium benzoate and potassium sorbate. Growth inhibition zones in disk diffusion method were between 17 mm and 20 mm. The antioxidant activities were between 134.4 and 188.5 mg gallic acid equivalents per gram of dry sample. Changes of weight, color, and pH were not significantly different from the untreated control sample. In sensory evaluation of the extract appearance (3.8 ± 1.5), flavor (4.9 ± 1.2), texture (5.2 ± 1.8), taste (4.5 ± 1.8) and overall preference of the RTE vegetable (4.5 ± 1.6), were evaluated.

Significance: These data suggest that GAC extracts may be selected as a candidate for natural preservatives of RTE vegetables. However, they might need masking agents for reducing the astringent taste of GAC extracts. Currently, more detailed study in sensory evaluation is going on in our laboratory.

P1-91 Inactivation of Superdormant Spores of *Bacillus weihenstephanensis* with Ozone

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Introduction: Psychrotolerant sporeformers such as *B. weihenstephanensis* are becoming increasingly recognized as microbiological hazards in refrigerated foods, specifically with the increased demand for refrigerated processed foods of extended durability. Aqueous ozone has been demonstrated to have a high potential to kill spores, however, the mechanism through which spores are killed by ozone is not well understood. Killing of spores by ozone is hypothesized to occur by causing damage to the spore's inner membrane. uperdormancy is hypothesized to occur due to the lack of expression of germinant receptors located on the spore's inner membrane.

Purpose: The purpose of this study was to determine the effects of aqueous ozone treatment on superdormant spores of *B. weihenstephanensis*.

Methods: Superdormant spores at an O.D. 600nm of 1 (2 mL) were treated with 190 ppm ozone in 48 mL sterile water. Samples were taken at 0, 30 s, 1, 5, 10, and 20 min time intervals and diluted 10% sodium thiosulphate to inactivate ozone. Spore pellets were centrifuged and washed with sterile water. Samples were further diluted in Tryptic Soy Broth, pour plated in Tryptic Soy Agar, and incubated for 48 h at 37°C. Superdormant spore recovery was determined by comparing standard plate counts from ozone-treated samples with nontreated samples.

Results: After 30 s of treatment with aqueous ozone 100% of superdormant spores were recovered and no spore inactivation was observed until 5 min. A significant reduction in recovery ($P = 0.0239$; $P < 0.05$) did not occur until after 10 min of treatment with 12.5% recovery. After 20 min of treatment time, only 5.5% of superdormant spores were recovered.

Significance: This study indicates that inactivation of superdormant spores of psychrotolerant species is possible with high levels of ozone.

P1-92 Sanitization of Pistachios Using Heat and Gaseous Ozone Combination

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Introduction: Recent salmonellosis outbreaks involving nuts have raised concerns about the safety of these products. Different technologies have been evaluated in order to determine feasibility for utilization against *Salmonella* on nuts. Since gaseous treatment with polypropylene oxide is currently used in the industry, ozone gas is a good candidate for integration into processing practices.

Purpose: The purpose of this study was to determine if including a gaseous ozone treatment in pistachio processing will be effective in reducing *Salmonella* on in-shell nuts. Additionally, *Enterococcus faecium* was evaluated as a *Salmonella* surrogate while determining the effectiveness of non-thermal processes.

Methods: Pistachios were inoculated with *E. faecium* or *Salmonella* Enteritidis. Treatments consisted of heat (5% brine at 70°C for 10 minutes), ozonation (160 g/m^3, 12.5 psig, 30 minutes holding time), heat followed by ozonation or ozonation followed by heat. The heating/brining step resembles a step commonly used in pistachio processing. Populations of the inoculated bacterium were determined before and after treatments using appropriate plating techniques.

Results: Results show that there is a significant log reduction of *E. faecium* on pistachios when treated with various methods. However, heat alone, heat followed by ozonation, and ozonation followed by heat, showed similar reductions (2.0, 1.9, 1.9 log reductions, respectively). Conversely, various treatments inactivated *Salmonella* Enteritidis to a greater extent than that observed with *E. faecium*, and the combination treatments were more effective than using heat alone (heat alone, 4.0 log; heat followed by ozonation, 5.5 log; and ozonation followed by heat, 4.6 log). For both bacteria, the application of gaseous ozone alone showed little reduction (0.5 log for *E. faecium* and 0.2 log for *Salmonella*).

Significance: The data obtained suggest that *E. faecium* is not a suitable surrogate for *Salmonella* when evaluating non-thermal processing, such as ozone, because the trends are not comparable. Including ozone in nut processing contributes to an effective combination treatment targeting *Salmonella* populations.

P1-93 Influence of Lactate and Acetate Salt Adaptation of *Salmonella* Typhimurium on Acid Resistance to Simulated Gastric Fluid at pH 2.0

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Introduction: Acetate and lactate salts are widely used as antimicrobials in poultry and meat products. However, studies have shown that exposure to these organic acid salts might induce adaptive responses in foodborne pathogens which allow them to survive the normally lethal treatment conditions and increase the likelihood of a foodborne outbreak.

Purpose: The aim of the present study was to determine the survival of *Salmonella* Typhimurium adapted with sodium lactate (NaL), potassium lactate/sodium acetate mixture (KL/NaA) and sodium acetate (NaA) in simulated gastric fluid (SGF, pH 2.0).

Methods: NaL-, KL/NaA- and NaA-adapted cells were prepared by incubating in tryptic soy broth (TSB) containing these salts at 5, 5 and 3% concentration levels, respectively, for 24 h at 37°C. The modified-Gompertz model was used to compare the growth kinetic parameters of adapted cells: lag phase duration (LPD) and maximum growth rate (MGR). The acid resistance of adapted cells was determined by incubating in SGF at 37°C.

Results: Adapted cells had longer LPD (NaL = 6.25, KL/NaA = 3.24, NaA = 5.78) than non-adapted cells (1.99 h). MGR was significantly ($P < 0.05$) slower in all adapted cells (NaL = 0.551, KL/NaA = 0.655, NaA = 0.392) than non-adapted cells (MGR = 0.919 log CFU/ml/h). The acid resistance of KL/NaA-adapted cells (D-value = 89 s) was not significantly ($P > 0.05$) different from non-adapted cells (D-value = 90 s). NaL-adapted cells (D-value = 48 s) were more susceptible to low pH environment of SGF, whereas NaA-adapted cells (D-value = 174s) showed enhanced acid resistance compared to non-adapted and other adapted cells.

Significance: These results suggest that the adaptation of *S.* Typhimurium to sublethal concentrations of NaA (sodium acetate) could enhance its ability to survive during stomach transit, and may increase the risk of a *Salmonella* outbreak.

P1-94 Intracellular Free Iron and Its Potential Role in Ultra-high Pressure Induced Inactivation of *Escherichia coli*

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Introduction: Ultra-high pressure (UHP) processing is being implemented as a novel food preservation technique, and the mechanism of microbial inactivation by pressure needs to be understood. Previous work revealed that expression of iron-sulfur cluster ([Fe-S]) biosynthesis genes was significantly affected by UHP treatment. The hypothesis is that UHP induces the increase of intracellular free iron, which leads to DNA damage and cell lethality.

Purpose: The objectives of this study were to (i) determine the intracellular free iron of *Escherichia coli* before and after high pressure using electron paramagnetic resonance (EPR) spectroscopy and (ii) investigate the effect of extracellular iron status [deprivation and overload, achieved by adding iron chelators 2,2′-dipyridyl (DIP) and $FeSO_4$ in LB broth] on the barotolerance of *E. coli*.

Methods: E. coli were inoculated in Luria-Bertani broth and incubated at 37°C aerobically or anaerobically. DIP and $FeSO_4$ were added to adjust the extracellular iron concentration as required. Cells grown to stationary phase were collected and then UHP treated. Iron was measured thereafter at 77 K on a Bruker ESP 300 X-band spectroscopy.

Results: For *E. coli* grown aerobically, concentrations of free iron were significantly elevated from 1.1×10^4 (no treatment) to 3.4×10^4, 5.1×10^4, and 1.2×10^5 (iron atoms/cell) after 300, 400, and 600 MPa treatments, respectively. However, for *E. coli* grown anaerobically, iron decreased from 9.6×10^4 (no treatment) to 5.2×10^4 (iron atoms/cell) after 500 MPa treatment. These results suggested that the increase in intracellular free iron is correlated to pressure-induced inactivation of *E. coli* grown aerobically, while the inactivation of *E. coli* grown anaerobically is achieved through a different mechanism. Additionally, for *E. coli* grown aerobically, iron-overload conditions produced cell populations consistently sensitive to UHP treatment (500MPa), regardless of the concentration of added iron. On the contrary, iron deprivation significantly increased barotolerance of *E. coli*.

Significance: This study provides a new perspective to elucidate the underlying mechanism of microbial inactivation induced by UHP, which will also aid in the development of more potent UHP-based processes for effective killing of *E. coli* and related organisms.

P1-95 High Hydrostatic Pressure Effect on *Escherichia coli* Heat-stable and Heat-sensitive Lux Proteins

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Introduction: The mechanism for microbial inactivation by high hydrostatic pressure (HHP) is an ongoing study. It has been suggested that pressure promotes protein denaturation which represents one of the main targets for bacterial viability loss.

Purpose: The purpose of this work was to monitor *in situ* the effect of HHP on the expression of two different lux cassettes on *E. coli* cells.

Methods: A high-pressure system connected to a photomultiplier tube was constructed to monitor bacterial metabolic activity. *E. coli* PL lux, expressing *lux* genes from *P. luminescens* and *E. coli* VF lux expressing *lux* genes from *V. fischeri* were exposed to cyclic treatments of 69, 103 and 138 MPa at 25°C for 30 minutes.

Results: Under pressure, bioluminescence decreased as % of initial light value to 17, 15 and 12% at 69 MPa; 4, 5 and 4% at 103 MPa and 6, 5, and 5% at 138 MPa within the 1st, 2nd and 3rd pressure cycle for *E. coli* PL lux. Upon decompression, light emission increased as compared to the initial light value to 72, 57 and 52% at 69 MPa at the 1st, 2nd, and 3rd cycle. In the case of *E. coli* VF lux, light decreased as % of initial value to 19, 16 and 15% at 69 MPa; 7, 7 and 7% at 103 MPa and 5, 5 and 5% at 138 MPa within the 1st, 2nd and 3rd compression cycle. *E. coli* VF lux showed a light recovery in % of initial light value equal to 70, 58 and 45% at 69 MPa for the 1st, 2nd and 3rd decompression cycle.

Significance: The results suggest a correlation between pressure and thermal stability being heat-stable lux proteins in *E. coli* PL lux more stable to pressure treatments than heat-sensitive lux proteins in *E. coli* VF lux.

P1-96 The Survival of Acid-adapted Pathogens in Maple Syrup with and without Preservatives during Storage at 10 and 20°C

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Introduction: Validation of food processes and products continue to be a challenge for food processors. Designing a study using parameters appropriate for the product type and conditions is essential to ensuring the safety of these foods and to protect the public health.

Purpose: To evaluate the survival of acid-adapted pathogens (*Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli* O157:H7) in the presence of preservatives in acidified syrup (pH range 4.2 – 4.5) stored at two different temperatures: 10°C (50°F) and 20°C ± 2°C (68°F) for 7 days.

Methods: The internal surface (bottom) of plastic syrup bottles (n =144) were spot inoculated with ~0.1 ml of 10^7 CFU/ml of acid-adapted organisms. The bottles were then filled with 150 ml of syrup, capped, and placed in incubators for storage at either 10 or 20°C for 7 days. Samples (n =12) were pulled at days 0, 1, 2, 3, 4, 5, 6 and 7 and plated on appropriate selective media.

Results: Levels of *E. coli* O157:H7 and *Salmonella* spp. decreased to below detectable levels in syrup with and without preservatives within the first 5 days of storage at both 10 and 20°C. While there was complete die-off of *L. monocytogenes* by Day 3 in syrup stored at 20°C, the organism survived past Day 7 of shelf life in both syrup types stored at 10°C. By Days 3 and 4, *S. aureus* was below detectable limits in both syrup types stored at 20°C; however, in syrup stored at 10°C, the organism continued to survive beyond Day 7.

Significance: The results of this study emphasize the importance of validating the microbial survival in food products using the correct parameters appropriate for the type of food, such as acid-adapted organisms for an acidified food, with storage at varying temperatures to ensure an accurate assessment of product safety.

P1-97 Osmotic Stress and Heat Resistance in *Escherichia coli*

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Introduction: The resistance of bacteria to a single environmental stress will often confer resistance to other stress conditions. It has been documented that accumulation of compatible solutes in response to osmotic stress contributes to increased resistance at high temperatures.

Purpose: This study compared two strains of *Escherichia coli*, *E. coli* AW1.7 exhibiting exceptional heat resistance, and *E. coli* GGG10 with nominal heat resistance. The effect of sodium chloride concentrations on heat resistance was determined and accumulated compatible solutes were identified and quantified.

Methods: Cell cultures were grown overnight in Luria Burtani broth with different NaCl concentrations. Samples were exposed to 60°C for up to 30 min followed by determination of cell viability. NMR was used to determine the composition and concentration of compatible solutes accumulated during growth.

Results: E. coli AW1.7 had similar heat resistance at 60°C when grown and heated in 2, 4% NaCl followed by a slight decrease at 6% NaCl. Cell counts were reduced by 1-2 logs after 30 min at 60°C. Heat resistance of *E. coli* GGG10 increased when cells were grown and heated in the presence of 4% NaCl with a cell count reduction of 5 logs after 15 min at 60°C. Further increase of NaCl concentrations to 6% NaCl caused a larger reduction in heat resistance than in *E. coli* AW1.7. When grown in the presence of 6% NaCl, *E. coli* AW1.7 accumulated more organic compounds compared to *E. coli* GGG10. Glucose, trehalose, lysine, and arginine were present at 1.3 and 0.7, 0.2 and 0.04, 0.6 and 0.4, 0.5 and 0.4 mM in *E. coli* AW1.7 and GGG10, respectively.

Significance: This study is the first to determine the solutes accumulated by two strains of *E. coli* with different heat resistance. Understanding the impact of stress from multiple hurdles on the survival of bacteria could provide valuable insight for the development of effective intervention strategies in food production.

P1-98 Effects of Combination of Hydrostatic Pressure, Amino Acids and Temperature on Germination of *Clostridium sporogenes*

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Introduction: High hydrostatic pressure (HHP) is known as a germinating factor for *Bacillus* spores, thus HHP followed by pasteurization can be a control measure for a broad range of *Bacillus* species. The effect of HHP on *Clostridium*, however, is still unclear.

Purpose: The purpose of this study is to evaluate the effect of HHP on germination of *Clostridium* spores in combination with the other germinating factors, amino acids and heat.

Methods: Spore suspensions of *C. sporogenes* NBRC 14293 supplemented by 1 to 80 mM amino acids were treated under 100, 200 and 400 MPa at 30 to 70°C, and then heated at 80°C to kill germinated spores.

Results: Though HHP seldom facilitated germination of the spores in the absence of amino acids, the presence of Ala, Cys, Ser, Gly, Met, Leu and Phe significantly enhanced the germination when combined with HHP. Cys and Ala markedly reduced viable spores by more than 4-log when pressurized at 100-200 MPa at 45°C. The effect of 200 MPa was greater than 100 and 400 MPa. The germination under 200 MPa was facilitated by increasing the amino acid concentration and the temperature when pressurized. Ala enhanced the effect of HHP in the widest range of concentrations, and the spores were successfully killed by 2-log even at 1 mM by the pasteurization following the pressurization.

Significance: It was clearly demonstrated that spores of *C. sporogenes* could be germinated by HHP by adding some amino acids and suggested an effective control measure for *Clostridium*.

P1-99 Removal of Viruses from Stainless Steel and Formica Food-contact Surfaces Using Various Cleaning Cloths

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Introduction: Each year enteric viruses, specifically human noroviruses, are identified as the dominant etiologic agent in foodborne disease outbreaks reported in the United Sates. The point of contamination may be traced back to the food handler, food preparation environment or source of production (i.e., farm or manufacturing environment). In order to control the transmission of viral pathogens in the food preparation environment, common methods used for the cleaning and decontamination of food contact surfaces need to be investigated for their efficacy against viral pathogens.

Purpose: In this study, 5 different cleaning cloths were evaluated for the ability to remove viruses from stainless steel and solid formica surfaces. The cleaning cloths included two different cellulose and cotton sponge sheets, one microfiber cloth, one non-woven cloth and one cotton terry bar towel. In addition, 2 different surrogates (murine norovirus (MNV) and MS2) for the study of human noroviruses and 1 surrogate (PRD1) for the study of human adenoviruses were evaluated.

Methods: Initially, a method was optimized for the recovery of the bacteriophages MS2 and PRD1 as well as MNV from the surfaces and the cleaning cloths. Following optimization, a mixture of the viral surrogates (10^5 to 10^6 PFU total) was inoculated onto either a 3 x 3 in. area of formica or stainless steel and allowed to dry completely. After drying, each area was wiped with one of the five cloths ($n = 6$ for each cloth-surface pair), and both the surface and cloth were processed using an elution method to recover viruses in order to determine removal of viruses by each cloth. Removal of viruses was determined by standard plaque assay methods.

Results: No difference was detected in the overall reduction of viral surrogates between the two surfaces following wiping with cleaning cloths (e.g., cleaning cloths performed the same on both formica and stainless steel). Four of the cleaning cloths removed between 10^2 to 10^4 PFUs while the microfiber removed an average of ≤ 10 PFUs (P-value < 0.05). Though not a statistically significant difference, the cellulose-cotton sponge sheets tended to remove more PRD1 as compared to the other 3 cloths. This trend was not seen for the other viral surrogates.

Significance: The findings of this study indicate that cleaning cloths composed of certain materials may be a valuable interim cleaning tool in the food preparation environment when time does not permit the use of a sanitizing agent. However, these should not replace environmental surface sanitizers such as household bleach which have been shown to be very effective against the inactivation of viruses.

P1-100 *Escherichia coli* O157:H12 Demonstrates Increased Ability to Attach to Abiotic Surfaces Compared with *E. coli* O157:H7 and O1:H7 Isolates

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Introduction: The attachment of *Escherichia coli* to abiotic materials is of concern to the food industry. Investigating the mechanisms of attachment is therefore important in order to control this organism.

Purpose: To investigate the role of different *Escherichia coli* flagellar H antigens in hydrophobicity and attachment to glass, Teflon and stainless steel (SS).

Methods: Δ*fliC* knockout mutants were constructed in single strains of *E. coli* O157:H7, O1:H7 and O157:H12. FliC expression was restored in Δ*fliCH12* and Δ*fliCH7* knockout strains by complementation with wildtype genes cloned in pGEM-T-Easy. Bacterial attachment was determined using epifluorescence microscopy. Hydrophobicity was determined using Bacterial Adherence to Hydrocarbons (BATH) and Contact Angle Measurements (CAM). Purified FliCH12 and FliCH7 proteins were prepared to functionalize Atomic Force Microscopy (AFM) colloid probes which were used to measure adhesion forces between FliC and all substrates.

Results: The loss of FliCH12 in the O157:H12 strain decreased its ability to attach to glass, Teflon and SS ($P < 0.05$). Complementation of Δ*fliCH12* restored the ability of this strain to attach to all surfaces to wildtype (wt) levels. The loss of *fliCH7* in the O157:H7 and O1:H7 strains did not always alter attachment ($P > 0.05$). Complementation of both O157:H7 and O1:H7 Δ*fliCH7* strains with *fliCH12* significantly increased attachment numbers to all surfaces for both Δ*fliCH7* strains in comparison to complementation with wt *fliCH7* ($P < 0.05$). BATH and CAM results indicated hydrophobicity was influenced by fliC expression but did not appear to play a role in attachment. Although no significant difference in adhesion force using AFM probes was observed between FliCH12 and FliCH7 probes ($P < 0.05$), differences in force curves suggest alternative attachment mechanisms for FliCH12 compared with FliCH7.

Significance: The results of this study indicate O157:H12 strains have an increased ability to attach to certain abiotic surfaces when compared to O157:H7 and O1:H7 strains, possibly due to differences in H antigens.

P1-101 Efficiency of Quorum-sensing Molecule Solutions in Biofilm Dispersal

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Introduction: Listeria monocytogenes, Escherichia coli O157:H7 and *Salmonella* Typhimurium are problematic foodborne pathogens that can be found in biofilms formed on foods and in food processing environments. Biofilms are known for their resistance to sanitizing agents and strenuous removal techniques. Therefore, improving upon typical sanitizing methods is of great importance.

Purpose: In this study we observed the effects of the signaling molecules, farnesol and tyrosol on *Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella* Typhimurium biofilms formed on surfaces commonly found on foods and food processing environments.

Methods: Each organism was individually grown in 96 well flat bottom plates, stainless steel chips and glass cover slips. Once biofilm formation occurred standardized crystal violet assays were performed in order to determine the efficiency of farnesol and tyrosol in biofilm dispersal. For each organism, solutions ranging from 1μM-1mM of farnesol and tyrosol were tested on all surfaces at a 0-10 minute time interval. YFP containing *Salmonella* were used to grow biofilms on spinach leaves. The leaves were treated with optimal biofilm dispersing concentrations and the effects were observed using fluorescent microscopy.

Results: For each surface and quorum sensing molecule solution a 1–3 minute treatment allotted for ~60% biofilm dispersal. Farnesol concentrations ranging from 1µM–50µM and tyrosol concentrations of 300µM–1mM were the most efficient solutions in biofilm dispersal. Biofilm dispersal could also be observed on the fluorescent microscope.

Significance: The results suggest that both farnesol and tyrosol are effective biofilm dispersing substances and could possibly be used in the field to reduce the amount of biofilm formation on foods and food processing equipment. The results also suggest that the quorum sensing molecules have a unique effect on the cells contained in the biofilms. More research is needed in order to find specifically how the quorum sensing molecules are affecting the biofilms.

P1-102 Characterization of Lactic Acid Bacteria on Biofilm Formation

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Introduction: Lactic acid bacteria (LAB) are known as health beneficial bacteria and have been used as flavor ingredients and biopreservatives in diary and fermented foods. However, there has been a limited number of studies on LAB as biosanitizers and their ability to form biofilm that protects food contact surfaces from contamination by pathogens. In previous studies, three LAB, *Pediococcus acidilactici*, *Lactobacillus amylovorus* and *Lb. animalis*, were isolated from ready-to-eat (RTE) meat and showed antilisterial effect.

Purpose: The purpose of this study was to evaluate their ability to form biofilm formation.

Methods: A hydrophobicity test was performed using hexadecane to determine the initial attachment. Based on this test, *Lb. animalis* exhibited the highest hydrophobicity with 28.6% while *Lb. amylovorus* yielded only 8.53% hydrophobicity. The result showed strong correlation with microtiter plate assay using crystal violet staining to examine attachment and biofilm formation in PVC microtiter plate wells.

Results: Lb. animalis and *P. acidilactici* consistently showed higher attachment on PVC plate wells than *Lb. amylovorus* when incubated at 23° and 7°C for 1, 3 and 7 days. Biofilm formations on stainless steel coupons were tested with individual LAB strains (10^7 CFU/ml) as well as the combined culture. *P. acidilactici* 5.09 x 10^5 CFU/ml (3.77% of biofilm formation) and *Lb. animalis* 2.46 x 10^4 CFU/ml (0.34%) were observed to form biofilm after 3 days of incubation at 23°C while *Lb. amylovorus* did not exhibit any biofilm formation. Interestingly, 1.5 x 10^7 CFU/ml of combined LAB formed biofilms at inocula of 1.02 x 10^9 CFU/ml (15.0%).

Significance: Cocktail of LAB indicated potential biofilm formation, which may be an important property for LAB use in the food industry.

P1-103 Biofilm Reduction and Growth Inhibition Using Virulent *Bacillus cereus* Phages Isolated from Soil and Fecal Samples

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Introduction: Bacillus cereus is a Gram-positive aerobic or facultatively anaerobic, motile, spore-forming, rod-shaped bacterium that is widely distributed environmentally. *B. cereus* is associated mainly with food poisoning and is being increasingly reported to be a cause of serious and potentially fatal non-gastrointestinal-tract infections.

Purpose: In this study, we isolated and characterized virulent bacteriophages which have host specificity to *B. cereus* and applicated as a *B. cereus* reducing sanitizing agent.

Methods: Isolation of the phages was performed by plaque assay. After propagation of homogenized soil and fecal samples in *B. cereus* KCTC 1094, it was filtered by 0.25um syringe filter and carried out isolation of the plaques by different morphology. Analyzing of the host spectrum of the isolated virulent phages in *B. cereus* and *B. thuringenesis* reference strains and other isolated strains were performed by spot assay. The morphology of the phages, which have wide host spectrum, was performed by Transmission Electron Microscopy. Also, analyzing the proteomic pattern of phages was generated by SDS-PAGE using purified phage particles. Inhibition growth of *B.cereus* by treating each bacteriophage in LB broth was performed by UV spectrometer. Analyzing the reducing growth of *B. cereus* in rice flour when treating BCP-3 BCP-11 phages by viable counting. Control of the *B. cereus* biofilm by treating BCP-3, BCP-11 was confirmed by crystal violet dying assay.

Results: 19 virulent bacteriophages were capable of specifically infecting *B. cereus* from different soil samples and fecal samples by plaque assay. Each phage had different host spectrums in *B. cereus, B. thuringenesis* reference strains and *B. cereus* group isolates. The host spectrum of BCP-3 had wide in only *B. cereus* and BCP-11 had wide host spectrum in *B. cereus* group such as *B. cereus* and *B. thuringenesis*. Based on electron microscopy evident morphology, BCP-3 and BCP-11 belonged to the Myoviridae family and each phage had different SDS-PAGE protein patterns. The growth of *B. cereus* KCTC 1094 in LB media and 50% rice flour solution were inhibited when treated with BCP-3 and BCP-11. Also biofilm of *B. cereus* was reduced when treated with BCP-3 and BCP11 phages.

Significance: B. cereus is widely distributed in environments, so it can contaminate various kinds of raw foods and manufactured food products. Therefore, we could control effectively by applying *Bacillus cereus* virulent phages at the raw material stage of agriculture and livestock products.

P1-104 Isolation of Virulent Bacteriophages for Bio-control of *Listeria monocytogenes*

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Introduction: Listeria monocytogenes is a major foodborne pathogen that contaminates ready-to-eat foods from the environment. Different control strategies are currently used, but because of the widespread occurrence of this bacterium, additional measures are needed. Bacteriophage treatments have been actively investigated.

Purpose: The aim of this project was to isolate *Listeria*-specific phages from natural sources and evaluate their lytic ability against *L. monocytogenes*.

Methods: Raw sewage samples were screened to isolate lytic phages of *L. monocytogenes*. Phages were purified and characterized phenotypically using plaque assays, spot tests, and efficiency of plaquing (EOP) methods. Phages were tested against 24 strains of eight *L. monocytogenes* serotypes to determine host ranges. A lytic phage cocktail (N = 6) was evaluated against a random mix of *L. monocytogenes* strains (N = 5, approx. 4 log CFU/mL) to determine the lysis potential of these phages with different multiplicity of infection (MOI) values (1, 10, 100, 1000) and temperatures (4, 10 and 30°C) in trypticase soy broth (TSB).

Results: As many as 37 *Listeria*-specific phages were isolated from sewage samples collected at different times and locations. Twenty-three phages were able to infect from 33 to 100% of the strains belonging to different serotypes by spot-testing. None of the phages were effective against 3c serotype. LMD3, (EOP range: 0.75–1.86) was the most effective, broad-host range phage. Phage treatments were most effective at 10°C and 4°C in TSB, as viable cells were not detected (< 1 log CFU/mL) after 24 h at 100 and 1000 MOI. Phage cocktail completely lysed *L. monocytogenes* cells at 1000 MOI after 1 h compared with controls.

Significance: Novel, lytic, wide-host range *Listeria*-phages were isolated from natural sources which might be an effective alternative as natural agents for bio-control of *L. monocytogenes* in foods and food production facilities.

P1-105 Variability in Growth and Ochratoxin A Production by Single Spores of *Aspergillus westerdijkiae, A. carbonarius* and *Penicillium verrucosum* in Response to pH, a_w and Temperature

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Introduction: Studies investigating fungal growth and mycotoxin production commonly use high inoculation levels (e.g., ≥ 10^3 spores/g or mL). However, contamination of foods by lower numbers of spores, or even a single spore, is a more realistic scenario in 'real life' and should be preferred in order to evaluate the dynamics and variability of fungal growth and mycotoxin production. Such information is limited, especially for ochratoxin A-(OTA) producing fungi.

Purpose: The aim of the present study was to evaluate the growth and OTA production kinetics of single spores of *A. westerdijkiae*, *A. carbonarius* and *P. verrucosum* in response to pH, a_w and temperature.

Methods: Single spores of all three fungi were isolated using a 1:2 serial dilution protocol in microtiter plates based on calibration curves relating optical density (600 nm) to spore density. The whole experiment was carried out in Malt Extract Agar of different pH (3.5 & 5.5), a_w (0.99 & 0.94) and temperatures (10, 15, 20, 25 and 30°C). Fungal growth was determined by measuring the colony diameter of 40–60 spores, while 6–8 single spores were tested at each sampling time for OTA production by HPLC analysis.

Results: Changes in a_w and temperature caused greater variability in fungal growth rates and OTA production compared to pH. The distribution width of colony growth rates and times-to-visible growth of single spores significantly increased at growth-limiting conditions (i.e., a_w 0.94; T = 15°C; pH 3.5), indicating a greater variation in the fungal response. The produced OTA by spores grown under the same stressful conditions, showed even more marked variability than that observed for their growth. Specifically, the levels of OTA released from spores that gave growth, ranged from below to above the legislation limits of 2–10 μg/L; whereas, in certain cases (i.e., a_w 0.99; T = 20°C; pH 5.5 for *P. verrucosum*) no toxin was detected.

Significance: Such findings may provide important and realistic information with regards to dynamics of individual spores to grow and produce OTA under different pH, a_w, and storage temperature.

P1-106 Comparison of Three Plating Media for Enumeration of *Clostridium perfringens* in Various Foods

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Introduction: Clostridium perfringens is one of the common causative agents of foodborne illness in U.S. Many plating media have been developed for quantitative detection of *C. perfringens* in foods.

Purpose: In this study, three media (tryptose-sulphite-cycloserine agar, TSCA; Shahidi-Ferguson-perfringens agar, SFPA; and modified *Clostridium perfringens* Chromogen agar, mCPCA) were compared for their ability to recover *C. perfringens* in artificially inoculated food samples, including Korean traditional fermented food.

Methods: All food samples were purchased from retail outlets in Seoul. Twenty-five grams of samples (instant red soy soup, *Korean* traditional fermented food (Soy bean paste), powdered infant formula (PIF), roasted grain and spring onion salad) were spiked with appropriate dilutions of *C. perfringens* to generate optimal number of colonies (30–300) for counting on the plating media. Samples were put into 225 mL of 0.1% peptone water followed by homogenization for 60 s with a stomacher. From the homogenates, TSCA and SFPA were tested using the pour plating method with an overlay of the same medium while mCPCA was tested using direct plating method followed by incubating anaerobically at 37°C (TSCA and SFPA) or 44°C (mCPCA) for 18 h. Five suspicious colonies on each plate were sub-cultured for additional confirmation using a real-time PCR targeting the *cpa* gene. All processes were repeated three times for statistical analysis.

Results: The recoverability was significantly greater ($P < 0.05$) on TSCA (79.9%) and SFPA (73.7%) than on mCPCA (4.53%) in PIF, red soy soup (TSCA, 16.5%; SFPA, 14.6%; mCPCA, 2.4%), and roasted grain (TSCA, 32.4%; SFPA, 28.9%; mCPCA, 4.8%). The highest and significant ($P < 0.05$) recoverability was observed in TSCA (82.89%), followed by SFPA (68.13%), and mCPCA (2%) when used for spring onion salad. Interestingly, all three media showed poor performance in soy bean paste (TSCA, 0.6%; SFPA, 0.2%; mCPCA, 0%).

Significance: This study indicates that the conventional plating media such as TSCA and SFPA are more effective for quantitative detection of *C. perfringens* than mCPCA in various foods considering their recoverability and cost. However, all of the media used for this study failed to enumerate *C. perfringens* in soy bean paste, a traditional Korean fermented food. Development of new media for enumeration of *C. perfringens* in soy bean paste should be followed.

P1-107 Determining Evidence of Enteric Bacteria on Environmental Surfaces in Living Areas of a Military Post in Turkey

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Introduction: Environmental surfaces in living areas can become easily contaminated with enteric bacteria through everyday use. Evidence of enteric bacteria on environmental surfaces may indicate that cleaning and sanitization practices are not adequate or sufficient, which could potentially result in cases of foodborne illness.

Purpose: To determine the presence of enteric bacteria on select environmental surfaces in living areas of a military post in Turkey.

Methods: A total of 99 surfaces with an average area of 25 cm^2 were taken with cotton swabs. Surfaces included door handles (N=16), handsink faucet handles (N=28), dining tables (N=20), bar soap dishes (N=7), kitchen equipment (N=8), and dining hall equipment (N=15). Samples were analyzed for thermo-tolerant coliforms, generic *E. coli*, *Shigella* and *Salmonella* using Multiplex PCR method.

Results: Only four of the 99 samples were positive for *Shigella* and one of these four samples was also positive for *Salmonella*. All four samples were taken from a bar soap dish.

Significance: In Turkey bar soap is used much more frequently than is liquid soap. Bar soap that is in continuous use can become contaminated with bacteria from hands. Bar soap could be a potential source for foodborne pathogens. Liquid soap is far less likely to be a source of contamination. Therefore, liquid soap should be used in place of bar soap.

P1-108 The Analysis Results of Food Products Conducted by Food Control Detachments of Turkish Armed Forces

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Introduction: One important way to reach the goal of a healthy community is by providing safe food products to the people. Food and waterborne diseases cause frequent epidemics in crowded settlements. The epidemics resulting from these kinds of diseases have been reported especially in army units, refugee camps, day nurseries and schools.

Purpose: This descriptive study was conducted to evaluate the results of food analysis performed by Food Control Detachment Units, which are responsible to monitor water and foodborne diseases in Turkish Armed Forces.

Methods: Food Control Detachments examined 1542 food specimens. Of these, 1157 were examined to check adherence to the criteria of contract documents, while 425 were performed for routine examinations.

Results: Of these examinations 40.3%(622 specimens) did not meet the criteria of safe food. Of the total food specimens, 16.7%(258) contained a high number of all organisms, while 12.6% (195), 5.9% (91), 25.2 (390) and 12.7% (196) were found to contain *Escherichia coli*, *Staphylococcus aureus*, yeast and mold, and coliform bacteria, respectively. The first five commonly analyzed products had very high proportions of unsafe specimens; cheese (51.2%), phyllo (81.5%), chicken meat (49.1%), yogurt (32.4%) and jam (13.1%).

Significance: In conclusion, in crowded areas (especially barracks), regular inspections of critical safe control units for healthy food products are very important. Many undersized epidemics can be prevented by hazard analysis and monitoring the critical control points.

P1-109 Proficiency of Microbiological Laboratories Testing Infant Formula and Nonfat Dry Milk

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Introduction: Proficiency testing (PT) is an inter-laboratory comparison used to determine analytical performance. Infant formula (dry and liquid) and nonfat dry milk samples were provided to laboratories for quality indicator bacteria and food pathogen analysis.

Purpose: These food matrices were chosen due to new requirements for infant formula safety and the increased number of foodborne illnesses from dry products. Evaluating proficiency of food testing laboratories is important to ensure preparedness in addressing current food safety concerns.

Methods: Liquid infant formula samples were inoculated with bacterial cocktails at desired levels and then verified for recovery. Dry powder samples were prepared by inoculating 1 kg of continuously mixing dry matrix with concentrated bacterial cocktails using an ultrasonic atomizer. Bulk samples consisted of targeted combinations of dry inocula. The samples were mixed for 30 minutes with a sterilized commercial mixer in a walk-in biosafety cabinet. Bacterial composition and homogeneity were confirmed by repeated sample analysis.

Results: Participants reported quantitative results for quality indicators (aerobic plate count, total coliforms, fecal coliforms, and *Staphylococcus aureus* counts) and qualitative results for pathogens. Multiple methodologies were used for analysis, including cultural, biochemical, immuno-assays, and PCR. The following qualitative results are reported as percentages of correct sample analysis with total analysts in parentheses. Nonfat dry milk analysis: *Salmonella* Typhimurim 100% (35), *Cronobacter sakazakii* 50% (2), *Shigella flexneri* 33% (12), *Listeria monocytogenes* 100% (22), *Escherichia coli* O157:H7 100% (33). Liquid Infant formula analysis: *S.* Typhimurim 97% (109), *C. sakazakii* 75% (12), *S. flexneri* 74% (45), *L. monocytogenes* 77% (104), *E. coli* O157:H7 97% (72). Dry infant formula results: *S.* Typhimurim 93% (28), *C. sakazakii* 100% (16), *S. flexneri* 44% (16), *L. monocytogenes* 84% (25), *E. coli* O157:H7100% (25).

Significance: The results allow assessment of the analysts' proficiency in identification of pathogens from specific foods and provides a precursory inter-laboratory comparison for the powder inoculation method. Proficiency testing is a vital tool for the advancement of food safety.

P1-110 Environmental Health Specialist Network's Impact on Foodborne Outbreak Investigations in New York State 2001–2008

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Introduction: The New York State (NYS) Environmental Health Specialist Network (EHS-Net) program covers 34 counties with a population in excess of 4.3 million. Since 2000, when NYS became an EHS-Net site, Environmental Health Specialists have been located in the Central Office to enhance surveillance, coordination, and ensure complete reporting to the Centers for Disease Control and Prevention (CDC) for foodborne outbreaks occurring within the catchment area. With additional staff and focus on outbreaks, it would be anticipated that counties participating in EHS-Net would demonstrate improvements in the timeliness and completeness of reporting foodborne outbreaks compared to the rest of the State (23 counties and a population > 6.8 million), excluding New York City.

Purpose: The purpose of this study is to assess the impact the EHS-Net program has on foodborne outbreak investigations in New York State.

Methods: The New York State Department of Health (NYSDOH) maintains foodborne outbreak data in its Foodborne Disease Surveillance (FBDS) database established in 1980. A descriptive analysis was conducted to compare the time to close out an investigation and identification of etiology for foodborne disease outbreak investigations occurring in counties participating in EHS-Net with counties outside the EHS-Net catchment area. Analysis was conducted using Microsoft Excel for calendar years 2001 – 2008.

Results: Over the eight year period, the average length of time between date of first onset for an outbreak investigation and receipt of the CDC 52.13 final report by the State was 13 weeks in EHS-Net counties versus 19 weeks in non EHS-Net counties. EHS-Net counties had a confirmed etiology in 66% (81/123) of foodborne outbreaks, compared to 57% (94/164) in non EHS-Net counties.

Significance: By providing funding for staff whose responsibility is to collaborate with epidemiologists and laboratorians, the Environmental Health Specialist program is likely to have a substantial impact on outbreak investigations and food safety. This publication was supported by Grant/Cooperative Agreement Number 1U01EH000701-01 from the Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC.

P1-111 Food Safety Knowledge among Restaurant Food Handlers in Northern Italy

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Introduction: Data on restaurant food safety knowledge among food handlers in Italy is very limited. These data are useful in prevention of food poisoning.

Purpose: The purpose of this survey was to determine food safety knowledge gaps among restaurant food handlers in Italy.

Methods: During June to November 2010, an oral survey that included 42 knowledge questions was administered to 100 food handlers in 100 randomly sampled restaurants in Bolzano, Italy. Surveys were administered in Italian or German languages based on the preference of the participants.

Results: To interview 100 restaurant food handlers, 143 food handlers were approached (response rate = 69.9%). The mean age was 45 years (range 20 to 74 years), 85% were male, and 68% had attained no higher than high school education. Knowledge of temperatures for cooking of chicken, ground beef, and the range of the danger zone for pathogen growth was very poor (7%, 13%, and 0%, respectively). The overall knowledge score was 27.8 / 42 questions (66.2%). The statement "You can be sure food is safe to eat when it smells and tastes normal" was recognized as false by only 38% of the food handlers. Differences in food handler knowledge by language were also observed. German-speaking food handlers more often answered correctly that raw meat cannot be stored above ready-to-serve food compared to Italian-speaking food handlers (94% v. 54%, respectively; $P<0.05$).

Significance: We observed substantial food safety knowledge gaps among Northern Italian restaurant food handlers including language-specific differences. These gaps provide educational targets for intervention in preventing foodborne illnesses. These data will be shared with northern Italian sanitarians.

P1-112 The Role of Foodworkers in Foodborne Disease Outbreaks Associated with Restaurant Settings, United States, 1998–2008

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Introduction: Foodborne outbreaks are reported to the Centers for Disease Control (CDC) by local, tribal and state public health departments. Examining restaurant-setting outbreaks is important for improving consumer safety and evaluating current food safety regulations.

Purpose: The objective was to examine food commodities, etiologies, and contamination factors associated with restaurant settings for foodborne outbreak investigations reported to the CDC from 1998 through 2008.

Methods: Data were obtained from CDC's Foodborne Disease Outbreak Surveillance System for outbreaks where food had been prepared in a restaurant or deli. Implicated foods were grouped into one of 17 commodities, if all ingredients belonged to that commodity. Outbreaks were categorized by etiology into those due to chemicals/toxins, those typically having an animal reservoir, those typically having a human reservoir, and those for which the reservoir is environmental/uncertain.

Results: A total of 7,965 restaurant- or deli-associated foodborne outbreaks were analyzed (102,923 illnesses, 3,519 hospitalizations, 62 deaths). A suspected or confirmed etiology was reported for 4,341 (55%) outbreaks. Human reservoir etiologies accounted for 47% of outbreaks with a reported etiology. Norovirus was the most common etiology (93% of human reservoir outbreaks), and leafy vegetables were the most frequent single-commodity food implicated (n=112, 38% of single-commodity outbreaks). Information implicating foodworkers as the contamination source was provided for 12% of restaurant-associated outbreaks, accounting for 25% of illnesses (n=26,032). In most of these outbreaks (76%), laboratory and/or epidemiologic evidence indicated that a foodworker contributed to contamination. Among the 1,162 (15%) outbreaks associated with human reservoir etiologies that had information about contributing factors, the most common factors were food handling by an infected person (56%) and bare-handed contact by a foodworker (41%). Foods with raw or lightly cooked ingredients were implicated in 43% of foodworker-contaminated outbreaks that reported food preparation methods.

Significance: These results suggest that human pathogens, especially norovirus, and foodworker contact with ingredients consumed raw are important in restaurant-associated foodborne outbreaks. Additional guidance and sick-leave policies for restaurant managers and foodworkers could decrease risk.

P1-113 Estimating the Burden of Foodborne Illness in Japan Using Web-based Survey Data for Extrapolating Estimates in Miyagi Prefecture to the Whole of Japan

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Introduction: We have been estimating the real burden of diarrheal diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Miyagi Prefecture, Japan based on laboratory-confirmed cases and estimated multiplying factors.

Purpose: The purpose of this study was to estimate the burden of foodborne illness associated with the above three pathogens in Japan using a cost-efficient method in extrapolating the estimates in the Miyagi Prefecture to the whole of Japan.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories in the Miyagi Prefecture from April 2005 to March 2008. The stool submission rate and physician consultation rate were estimated from two population telephone surveys conducted in the same prefecture. Each factor was introduced in our Monte-Carlo simulation model as a probability distribution. These rates were separately estimated by using a web-based survey and compared between the whole of Japan and Miyagi Prefecture, which were further used to extrapolate Miyagi estimates to the whole of Japan.

Results: The physician consultation rate and the stool submission rate calculated from the web-based survey were 27.3% and 6.7% for Miyagi Prefecture and 19.3% and 3.6% for the whole of Japan, respectively. We extrapolated the estimated illnesses in Miyagi Prefecture to the whole of Japan using the population rate, understanding that the extrapolation was not at least an overestimate. The estimated mean annual numbers of foodborne illness for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus*, were approximately 1.3–1.6 million, 140–254 thousand, and 18–83 thousand in Japan during 2005–2008, respectively. The numbers of reported foodborne illness due to these pathogens in the same period were approximately 2–3 thousand, 2–3 thousand and 0.1 - 2 thousand, respectively.

Significance: These data revealed a significant difference between our estimates of burden of foodborne illness and the reported foodborne disease cases, suggesting the need to supplement current statistics with burden estimate research.

P1-114 Effects of Corn-based Distillers' Grain (DG) Inclusion into Feeding Rations on the Burden of *Escherichia coli* O157:H7 in Commercial Feedlot Settings

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Introduction: Use of corn-based distillers' grains has been associated with an increased prevalence of *Escherichia coli* O157:H7 in feedlot cattle. However, the research has been conducted in small-pen research settings and the relevance to real-world commercial settings is unclear.

Purpose: To quantify the potential relationship between the use of distillers' grains co-products and *E. coli* O157:H7 pathogen load in real-world feedlot settings.

Methods: Ten commercial feedlots were split into cohorts in which finishing diets contained either high DG (> 15%) or low DG (< 8%). Feedlots were sampled on 4 occasions from June thru October of 2010 with approximately 6 weeks between each sample collection. At each feedlot visit, 4 pens of cattle within 3 weeks of harvest were enrolled and 24 freshly voided fecal pats were collected from each pen. Ten gram samples were placed into 90mL of modified tryptic soy broth supplemented with novobiocin and enriched for 14 hours at 42°C. Enrichments were subjected to immunomagnetic separation, plated onto CHROMagar™ with novobiocin, and incubated for 18 hours at 37°C. Suspected colonies were confirmed via agglutination.

Results: Of 3,840 samples, *Escherichia coli* O157:H7 was recovered from 16.7% of the samples. Controlling for within-feedlot and within-pen clustering, adjusted prevalence was 14.3%, indicating some evidence of within-group clustering. Variation within feedlots over time was substantial;

for example, within one feedlot, mean sample-level prevalence ranged from 7.6% to 42.7%. Prevalence within the 4 sampling rounds was 19.9% (sample window = 28JUN to 12JUL), 21.0% (AUG), 14.1% (SEP) and 11.7% (OCT). Significant interaction was observed between DG use and sampling round ($P < 0.01$). Prevalence among high use was greater than those with low use for sampling Rounds 1, 3 and 4 but not different for Round 2. Averaged across time, prevalence among high and low users was 21.4% versus 7.3%, respectively, and vary with statistical model.

Significance: These data indicate a higher prevalence of *Escherichia coli* O157:H7 associated with higher inclusion rates of DG into feeding rations in commercial feedlot settings.

P1-115 A Semi-quantitative Methodology for *Escherichia coli* O157:H7 in Bovine Feces

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Introduction: Improved, accurate, and affordable methods to quantify *E. coli* O157:H7 in bovine feces are needed. To date, several methods have been proposed but vary from relatively labor-intensive to expensive.

Purpose: To compare a semi-quantitative method with direct plating and to assess changes in concentration in samples stored over time at 4°C.

Methods: Freshly voided fecal pats collected from commercial feedlots were confirmed to be naturally infected with *E. coli* O157:H7 detection in a side study. Positive samples' (n = 331) immunomagnetic separation (IMS) plates were ranked (1–5) according to number of suspect colonies produced post incubation (1 =1–10, 2 =11–20, 3 = 21–30, 4 = 30 + 5 = Lawn). Confirmed positive fecal samples (1g) were then serially diluted and direct plated to CHROMagar™ with incubation for 18 hours at 37°C for quantification. A subset (n = 69) were stored at 4°C and subjected to re-assessment over a 10 week period, 11 samples of which were high ranked (rank = 4 or 5) and direct plated during each re-assessment.

Results: Concentration was associated with a quadratic rank term ($P < 0.01$). On average, ranks 4 and 5 were associated with quantifiable direct plating whereas ranks 1 through 3 were below the detection limit of direct plating. Mean values for ranks 1–5 were; 0.02, 0.0, 0.0, 0.43 and 3.17, respectively. In the extension study, positive detection by IMS had decreased 49% by Week 2, 52% by week 4, 67% by Week 6, 71% by Week 8 and 81% at Week 10. The ability to detect via direct plate decreased by 50% at Week 2 while overall count averages decreased by 99.99% by Week 10. Similarly, IMS rank averages decreased over the sampling duration.

Significance: These data indicate that the semi-quantitative IMS ranking method may be used as a convenient and repeatable proxy for quantification while decreasing costs and labor. These data also indicate fecal samples should be analyzed sooner than 2 weeks at 4°C.

P1-116 Culture-independent, Metagenomic-based Characterization of *Campylobacter jejuni* on DNA Isolated Directly from Complex Samples

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Introduction: The characterization of *Campylobacter jejuni* strains in epidemiological studies is commonly performed on strains isolated from culture of complex samples. However, studies have shown that significant numbers of viable *C. jejuni* are not recovered by the selective culturing schemes applied commonly.

Purpose: To circumvent this bias, a culture-independent metagenomic-based method for studying the diversity of *C. jejuni* isolates in poultry boot-swab samples was developed.

Methods: The method involved an end point dilution PCR and sequencing of the *flaA* SVR in DNA extracted directly from poultry boot-swab samples. The end point dilution PCR was used to isolate coexisting *flaA* sequence variants by diluting the extracted boot-swab DNA to the point of lowest detection. The *flaA* SVR sequences types obtained from the metagenomic-based method were compared to the *flaA* SVR sequence types obtained from sequencing up to 10 cultured isolates from the same samples. Three boot-swab samples originating from different flocks of free ranging organic geese were analyzed in parallel by the two methods.

Results: For the first sample, the metagenomic method resulted in 8 different *flaA* SVR sequence types, while sequencing cultured *C. jejuni* colonies from the same sample, resulted in three different *flaA* SVR sequence types. Only one of the sequences was found using both methods. For the second sample, the metagenomic method resulted in one *flaA* SVR, while the culture based method resulted in two *flaA* SVR sequence types, both of them different from the one detected using the metagenomic method. For the third sample, three *flaA* SVR sequence types were found using both methods, but one more *flaA* SVR type was found using the metagenomic method.

Significance: The observed *C. jejuni* diversity in the analyzed boot-swab samples was significantly different for the two methods, suggesting that the selective culture-based methods, on their own, do not result in a complete picture of the *Campylobacter* spp. diversity in complex environmental samples. Using a combination of both methods in epidemiological studies could give a more comprehensive picture of the diversity of *C. jejuni* isolates and aid in the resolution of the infection dynamics. The metagenomic-based method studied here paves the way for the development of a new, faster and more cost effective way for characterizing microbial pathogens in complex environmental samples.

P1-117 Persistence of Verocytoxogenic *Escherichia coli* in a Pasture-based Beef Cow-calf Cohort

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Introduction: Cattle are a major reservoir of verocytoxogenic *Escherichia coli* (VTEC) and repeated outbreaks of human illness have been linked to consumption of beef. The majority of studies on VTEC in cattle populations have focused on screening for the presence in feedlot production systems, which use different production practices with different risk factors compared to pasture systems. The few pasture production system studies that exist are mainly searching for presence of the pathogen at a single time point, and do not determine the duration and transmissability of the strain to other cattle in the system.

Purpose: Determine duration and transmission of VTEC shedding in cows in a cow-calf pasture production model. The virulence potential for humans was assessed by screening for 5 key pathogenicity genes.

Methods: Fecal samples from pastured cows were sampled pre-pregnancy, and cows, calves and environmental samples were taken weekly after birth for two months. Each sample was enriched for *E. coli* and screened for VTEC using PCR. Virulence potential was assessed using PCR to detect Shiga toxin (*stx*1, *stx*2), intimin (eae), enterohemolysin (*ehxA*) and an adhesion (*saa*).

Results: Members of this herd were positive for VTEC in samples obtained in fall 2008, spring 2009 and fall 2010 (12/90). Other cows were positive for VTEC during one sampling period (15/90). Presence of virulence genes were different for cows housed in different paddocks. No calves were identified as positive, even from shedding cows.

Significance: Identification of potential contamination factors in cow-calf cohorts will allow identification of risk factors, specifically within pasture production facilities. Control practices can then be tailored to specific reservoirs, such as reducing specific wildlife populations, therefore reducing product contamination while increasing beef safety in the eyes of the consumer.

P1-118 Outbreaks of *Salmonella* Associated with Beef, United States, 1998–2008

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Introduction: Beef is frequently implicated as a transmission vehicle in foodborne disease outbreaks, but outbreaks of *Salmonella* infection associated with this food commodity have not recently been summarized.

Purpose: To describe the frequency and characteristics of outbreaks of *Salmonella* linked to beef.

Methods: The Centers for Disease Control and Prevention (CDC) conducts surveillance for foodborne disease outbreaks. We reviewed data for *Salmonella* outbreaks where beef was reported as the food vehicle during 1998–2008. We analyzed outbreak frequency and size, number of hospitalizations and deaths, *Salmonella* serotypes, implicated cuts, settings of food preparation, and reported contributing factors.

Results: From 1998-2008, 39 *Salmonella* outbreaks were reported with beef as the food vehicle, causing 1161 illnesses, 114 hospitalizations, and 1 death. At least one outbreak was reported each year (range, 1–9). The average outbreak included 30 cases (range, 2–155). Sixteen *Salmonella* serotypes were reported in the 34 outbreaks for which the serotype was known, most commonly Newport (7 outbreaks), Enteritidis (5), and Typhimurium (5). Implicated cuts included ground beef (12 outbreaks, 31%), steak or roast (7, 18%), another cut (8, 21%), or unknown (12, 31%). For ground beef outbreaks, beef was prepared in a private home in 8 (75%). For non-ground beef outbreaks, beef was prepared in a restaurant or deli in 13 (48%) and in a private home in 6 (22%). The most commonly reported contributing factors were insufficient time or temperature during cooking (e.g., eating pink/raw ground beef) and contaminated raw product.

Significance: During these 11 years, outbreaks of *Salmonella* infection transmitted by beef occurred at least annually. Outbreaks were frequently associated with contaminated product prepared in consumers' homes. Understanding the epidemiology of *Salmonella* outbreaks in beef, including cuts of beef involved and settings where outbreaks occur can help to guide decisions about regulating *Salmonella* in beef and consumer education efforts.

P1-119 *Cryptosporidium* spp. in Wild Rodent Populations Adjacent to Produce Production Fields

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Introduction: Wildlife are increasingly considered as potential pathogen sources that transmit pathogens to food animals and contaminate pre-harvest produce. *Cryptosporidia* parasites infect a wide range of vertebrates including farm animals, wildlife and humans. The parasites cause waterborne and foodborne illness by contaminating water and foods.

Purpose: The purpose of this work was to determine the incidence and identify the potential role of wild rodents in *Cryptosporidium* transmission and contamination in the pre-harvest food production environment.

Methods: Between 2009 and 2010, fecal samples were collected from wild rodents in fields adjacent to cattle rangelands and leafy green blocks in one of California's major produce production regions. Rodents were live-trapped and released after collecting defecated fecal samples. Samples were screened for *Cryptosporidium* spp. oocysts using fluorescent microscopy. PCR products from positive samples were sequenced.

Results: A total of 265 fecal samples were obtained from 10 species of wild rodents. *Cryptosporidium* spp. oocysts were detected in 73 (27.5%) of the samples. For specific rodent species, *Cryptosporidium* spp. were detected in 0% (0/3) of Brush mouse, 33.3% (1/3) of California ground squirrel, 10.5% (4/38) of California parasitic mouse, 0% (0/2) of California pocket mouse, 32.8% (61/186) of Deer mouse, 16.7% (1/6) of Dusky-footed wood rat, 0% (0/1) of Harvest mouse, 0% (0/2) of House mouse, 33.3% (1/3) of Kangaroo rat, 0% (0/4) of Meadow vole and 29.4% (5/17) of undetermined species. Analysis of DNA sequences for *Cryptosporidium* species/genotypes determination is underway.

Significance: A considerably high incidence of *Cryptosporidium* spp. exists in the wild rodent population adjacent to rangeland and produce areas. This highlights the potential risk of transmission to food animals and contamination of pre-harvest produce. Findings suggested the need of developing agricultural and environmental strategies preventing wild rodents from transmission *Cryptosporidium* to food animals and contamination of pre-harvest produce.

P1-120 A Novel Approach to Predicting Lag Time of *Bacillus cereus* as a Function of Temperature, pH and Water Activity Using Logistic Regression

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Introduction: Controlling the factors affecting the lag time during growth of spoilage and/or pathogenic bacteria in prepared/processed foods with relatively short shelf lives plays a key role in ensuring the quality and safety of the food. Appropriate prediction of the lag time will lead to a reasonable shelf life and also to reduced waste.

Purpose: The objective of this study was to develop a probabilistic model to predict the lag time during growth of *Bacillus cereus* as a function of temperature, pH and salt concentrations using logistic regression. In addition, we aimed to develop a novel *B. cereus* growth kinetics model incorporated with the newly developed lag-time model.

Methods: B. cereus growth was evaluated in a nutrient broth adjusting the pH (5.0~6.5) and salt concentration (0.5~2.0%) under temperatures between 10 and 20°C. The optical density (OD) values were monitored continuously during incubation, and the lag time was determined by the changes in OD value. The lag time was modeled using a logistic regression procedure as a function of temperature, pH and salt concentration. The developed model for the lag time was combined with a logistic type growth differential equation to obtain entire bacterial growth kinetics under given conditions. Finally, the developed model was validated by the experimentally observed *B. cereus* growth data in 6 kinds of Japanese deli foods.

Results: The lag time of *B. cereus* was successfully modeled using logistic regression indicating a high percent concordant (95.8%). The lag-time model provided a probability distribution of the estimated lag time, and the observed lag times in the tested 6 kinds of deli foods were fallen in 95% confidence intervals. The *B. cereus* growth kinetics in the tested deli foods was accurately predicted overall using the combination of the developed lag-time model and logistic differential equation model, indicating small root mean squared errors of the predictions (~0.5 log CFU/g).

Significance: The lag-time model developed in the present study can be used to estimate not only the lag time itself but also the probability distribution of the lag time. In addition, the model allowed us to appropriately predict the *B. cereus* growth kinetics in various real foods taking into account the lag time.

P1-121 Development of a Predictive Modelling Tool to Simulate a_w of Food Products

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Introduction: The influence of water activity on microbiological and organoleptic properties of food is a well-known phenomenon. Several models have been described in order to calculate water activity of a solution as a function of its composition, but no available model exists for the prediction of the a_w of formulated foodstuff.

Purpose: The aim of this study is to develop a predictive software for the simulation of water activity as a function of the composition of food products.

Methods: Foods are considered to be made up by several phases (insoluble and soluble) with different physico-chemical properties, as well as different kinds of interactions with water. Calculation principles used are based on the modelling of the hygroscopic properties, as well as the interactions between water and each individual components of the considered food products: 1) water adsorption by insoluble components (like proteins or starch) is evaluated by sorption isotherms which are fitted by using Ferro-Fontan and Guggenheim-Anderson-de Boer (GAB) models and, 2) different models (Roa, 1998 Teng and Lenz, 1974) were studied to evaluate the interaction between solutes (like sugar, salt, amino-acids…) and water.

Results: Fitting model parameters were evaluated for each of the 230 components available on the database (including starches, protein concentrates, fibers, gums, flours, fat, egg products, salts, spices, meat, cereals, fruits, vegetables, preservatives antioxidants and emulsifiers...). Assuming thermo dynamical equilibrium, the computer program, developed on Matlab®, calculates the distribution of water between aqueous and insoluble components until the water activity is equal in both phases. Validation of the mathematical model was done on different types of matrices with a_w value range of 0.7 to 1 (bakery products, meat products, sauce and syrup). There were great correlations between predictive values and a_w value measured with a_w Aqualab a_w-meter (R^2 =0.94 for bakery products and R^2=0,88 for syrup). The mean error is 0.015 a_w.

Significance: This work presents a valuable tool for the simulation of a_w as a function of food formulation. This tool was developed for industrial and academic development in food conception and shelf life evaluation.

P1-122 The Risk Estimation of *Listeria monocytogenes* for Ready-to-Eat Food in Korea

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Introduction: Ready-to-eat (RTE) foods, which can be consumed without further cooking and/or reheating, can be considered potentially high-risk foods. Foodborne disease outbreaks associated with RTE foods have generally been related to contamination by *L. monocytogenes*, as they are usually prepared by hand and stored at low temperatures. The most popular RTE foods in Korea were monitored in this study, and a predictive growth model, frequency, consumption quantity, and dose-response model were assessed in order to estimate the risk of *L. monocytogenes* contamination in RTE foods in Korea.

Purpose: This study describes a quantitative microbial risk assessment (QMRA) model of Korean cases of listeriosis occurring due to the consumption of kimbab, smoked salmon and fresh-cut vegetables, taking into consideration the primary data acquired during the exposure assessment step. The results of this QMRA can be used by administrators to establish national regulations for the control of foodborne diseases.

Methods: We developed a one-dimensional risk assessment model to describe mathematically the annual risk of listeriosis associated with the consumption of RTE foods (kimbab, smoked salmon and fresh-cut vegetable products) in Korea. We monitored microbial contamination levels of RTE foods and frequency and consumption quantity for MRA.

Results: The mean and maximum number of cases of listeriosis arising annually due to consumption of kimbab, smoked salmon and fresh-cut vegetable products per ten million individuals were estimated as 5.8×10^{-2}, 2.5×10^{-3}, 2.5×10^{-2} and 5.49, 0.1, 2.08. These results indicated that the risk factors of regression sensitivities, from the retail to table pathway, could be applied to risk management. In the future, additional studies will be required to facilitate more realistic and accurate microbial risk assessments.

Significance: As the population of Korea numbers approximately 50 million, approximately 40 patients per year are expected to contract *L. monocytogenes* infections due to kimbab, smoked salmon and fresh vegetable intake. Moreover, considering the increase in the consumption of RTE foods due to their relative convenience and economic feasibility, the actual risk is expected to be somewhat higher than the estimated risk. Also, additional studies regarding selective evaluation on groups of extreme intake and sensitive consumption, as well as the development of dose-response models and statistical methods, will be required for more realistic and more accurate assessments of QMRA.

P1-123 Development and Validation of a Predictive Model for *Escherichia coli* O157:H7 in Spinach and Lettuce

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Introduction: Of 1,973 foodborne disease outbreaks reported in Korea from 2005 to July 2010, 277 (26.3%) were due to the pathogenic *Escherichia coli*. Lettuce and spinach are the most commonly implicated produce associated with *E. coli* O157:H7. Predictive models for *E. coli* O157:H7 in broth have been developed as a function of temperature, pH, and sodium nitrite. However, the growth model for *E. coli* O157:H7 on fresh produce has not been developed.

Purpose: The objective of this study was to develop predictive growth models for *E. coli* O157:H7 on spinach and lettuce as a function of temperature.

Methods: Raw spinach was blanched and lettuce was sterilized for one hour by UV-light to suppress background microorganisms. Blanched spinach and sterilized lettuce were inoculated with 100 µl of a two-strain mixture of nalidixic acid-resistant *E. coli* O157:H7(NCTC 12079 and ATCC 35150) and were stored at 7, 10, 17, 24, 30 and 36°C in two different laboratories, respectively. Lag time(LT) and specific growth rate (SGR) were used in square-root and Davey models as a function of temperature, respectively. Model performance was cross-evaluated for interpolation with the same strain and modeling method, but a different model food in each laboratory. Prediction bias (Bf) and accuracy (Af) factors were calculated.

Results: No significant differences were observed in the LT model above 24°C and in the SGR model below 17°C between spinach and lettuce. These data indicate that difference in spinach and lettuce as a model food did not significantly affect LT values at 30 and 36°C and SGR values at 24, 30, and 36°C. Bf of LT and SGR models was 1.02 and 0.99 for spinach and 0.91 and 0.80 for lettuce, respectively, with independent data for interpolation.

Significance: These models might be used in the development of tertiary models to quantify the effects of temperature on the growth of *E. coli* O157:H7 on fresh produce, but model performance must be validated with different types of fresh produce.

P1-124 Modeling the Effect of Temperature and pH on the Growth Rate of *Salmonella* on Cut Tomatoes

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Introduction: Outbreaks of salmonellosis associated with fresh cut tomatoes has been a recent food safety concern. When tomatoes are cut, *Salmonella* can be transferred from the skin to the flesh, which is a favorable growth environment.

Purpose: The manipulation of fresh cut tomato pH offers one possible means for *Salmonella* control in cut tomato products. The purpose of this research was to expand our existing research efforts on modeling *Salmonella* in fresh cut tomatoes by adding the additional variable of pH, resulting in a regression model able to predict *Salmonella* growth as a function of both pH and temperature.

Methods: Whole red, round tomatoes were dip-inoculated in a cocktail of *Salmonella* strains obtained from the CDC. These human isolates were from cases associated with prior salmonellosis outbreaks linked to tomatoes. Inoculated tomatoes were dried overnight and cut into slices and incubated at temperatures from 10 to 30°C in 5-degree intervals. The pH of the cut tomatoes was adjusted from 3.8 to 4.2 by the addition of 1% or 5% citric acid. Samples were enumerated by plate counts on XLT4 agar until *Salmonella* populations reached stationary phase. Growth rates were calculated using DMfit software.

Results: A plot of square root (SQRT) of the growth rate (GR) of *Salmonella* under various temperature and pH = 4.0 was linear with time, such that SQRT (GR) = 0.022T + 1.148 (R2 = 0.79), which is slower than the growth rate under tomatoes' natural pH (~pH = 4.4) found in previous studies. Moreover, *Salmonella* growth was suppressed at pH = 3.8 at all temperatures from 10 to 30°C.

Significance: The model of *Salmonella* in cut tomatoes created here is the first of its kind that provides a useful tool of estimating the risk posed by different degrees of temperature abuse and pH manipulation.

P1-125 A Quantitative Microbial Risk Assessment Model for Reducing the Incidence of Human Campylobacteriosis Due to Chicken Consumption

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Introduction: Food-safety policy trends have progressively shifted from hazard- to risk-based approaches. Risk analyses have become more integrated in developing modern food safety systems. Although *Campylobacter* is considered a major causative agent for foodborne illnesses in the U.S., it is overshadowed by higher-profile pathogens. Chicken consumption provides a source of relatively inexpensive animal protein in consumer diets, but is also considered the primary food vehicle for *Campylobacter*.

Purpose: More comprehensive and modifiable risk assessment models are warranted for use in food-safety systems. Using risk analysis, the annual health and societal impact of *Campylobacter*-associated chicken consumption in the U.S. can be measured and updated.

Methods: Quantitative microbial risk assessment models were developed to gain insight into the prevalence and concentration of *Campylobacter* spp. at various stages of poultry production, processing, retail, and consumer storage and handling. The pathogen levels were fit into a dose-response model that yielded estimations of annual health impacts of chicken product consumption. Health-impact estimations were separated by illness predictions of various levels caused by consumer-exposure. Health-impact estimations were linked to cost-of-illness predictors using nationally available data to demonstrate the societal burden caused by this pathogen-food-consumer interaction.

Results: Annual illnesses resulting from consumption of *Campylobacter*-contaminated chicken were estimated at 426,118 (90% CI 0.021-; 1.20-million). Additionally, 2,417 hospitalizations (90%-CI 111; 6,447), 450 cases of secondary infections (90%-CI 21; 1,180), and 89 (90%-CI 2; 284) deaths were also attributed. The socio-economic burden yielded approximately $1 billion per year.

Significance: Campylobacter-contaminated chicken consumption presents significant health- and socio-economic burdens in the U.S., as demonstrated with this quantitative risk assessment. Moreover, the marriage of utilizing commercially available risk assessment software with the accessibility of processing power available to end-users should yield more sophisticated quantitative risk-assessment models with fewer resource burdens than in the past. The use of risk-analysis systems will continue to change the culture of, and approaches, to food safety.

P1-126 Quantitative Risk Assessment for Campylobacteriosis in New Zealand by the Bayesian Belief Network Approach

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Introduction: New Zealand has the highest rate of reported campylobacteriosis cases in the developed world. Due to the large economic and health consequences of campylobacteriosis, intervention programs to reduce the disease rates are required to be designed and implemented. Quantitative microbial risk assessments (QMRA) are used to identify all the risk pathways in the food chain and to examine the most effective interventions to reduce the rate of foodborne illness. Many of these risk assessment studies were conducted by infeasible Monte Carlo approach.

Purpose: The purpose of this study was to conduct QMRA by Bayesian Belief Network approach which has many advantages such being an appropriate method in handling missing data, accounting for the uncertainty in all estimated parameters, enabling the combination of data with the experts' prior belief or knowledge; it allows back propagation of prior variables with the observed data, it assists understanding about underlying relationships between variables, it provides a method for avoiding overfitting of data, and it offers satisfactory prediction accuracy.

Methods: A simplified model was developed describing the entire food chain from farm to fork with all the variates, parameters, and variables of interest. The joint probabilistic distribution of these variates of interest was specified using successive conditional distributions. Microbiological data of two New Zealand poultry processing plants for the last two years were incorporated. The numerical computations were performed using WinBugs software.

Results: The Bayesian approach confirmed its strength in handling a complex and challenging situation (model), presenting a plausible estimation of the probability of contracting campylobacteriosis by poultry consumption and the probability of contracting campylobacteriosis from all other resources. The mean value of the estimated probabilities were close to the actual Surveillance data. Sensitivity analysis showed the significant role of consumer hygiene in reducing the outcome probability.

Significance: The results of this study provided an attractive and reliable tool for risk management to select the best and effective intervention (e.g., education of consumers) to reduce campylobacteriosis given the impossible to produce *Campylobacter*-free chicken.

P1-127 Survival of *Salmonella* spp. on Inoculated In-shell Pistachios Stored at -20, 4 and 23°C

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Introduction: Few data are available on the survival of *Salmonella* spp. on pistachios.

Purpose: The survival of *Salmonella* spp. on the surface of in-shell pistachios inoculated at different levels and stored at various temperatures was evaluated.

Methods: Raw and roasted in-shell pistachios were inoculated with three strains of *Salmonella* associated with foodborne outbreaks. The pistachios were inoculated at three levels (ca. 8, 5 and 3 log CFU/g) and stored under three temperature conditions (room temp, 23 ± 3°C; refrigerated, 4 ± 2°C); and frozen, -20 ± 2°C). *Salmonella* levels were enumerated at 0, 1, 2, 3 and 4 months.

Results: Salmonella levels declined slowly in raw pistachios, and in inverse relation to the temperature. The average reduction rates across three levels of inoculum (±standard deviation) for room, refrigerated and frozen temperatures were -0.17 ± 0.06, -0.09 ± 0.02 and -0.08 ± 0.06 log CFU/month, respectively. In contrast greater and more variable reduction rates were observed in roasted pistachios. The corresponding average

reduction rates for roasted pistachios for room, refrigerated and frozen temperatures were -0.56 ± 0.32, -0.62 ± 0.36 and -0.27 ± 0.10 log CFU/month, respectively.

Significance: The information developed in this study can be used in risk assessment modeling to account for reductions which might occur during the production of pistachio products, in distribution and after purchase by consumers when stored at various temperatures.

P1-128 Monte Carlo Estimation of the Efficacy of Spent Sprout Irrigation Water Testing

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Introduction: It is generally recognized that seed washing and sanitation are not sufficient to eliminate pathogens in sprouted seed products. Therefore, the FDA recommends that seed sanitation be combined with microbial testing of spent sprout irrigation water.

Purpose: We estimated how effective testing of spent sprout irrigation water testing is in providing protection in the event of incoming seeds having low levels of contamination.

Methods: A Monte Carlo model was constructed to estimate how effective testing of spent irrigation water would be at detecting 1 or more pathogenic organisms in a 10 lb charge of seed in one quadrant of a sprouting chamber by testing of spent sprout irrigation water after 48 h of sprouting (the accepted industry practice). The model included as factors the time to the appearance of the seed radicle, wash cycle interval, wash volume, organism growth and partition between seeds and spent irrigation water. The number of organisms reaching the spent irrigation water after 48 h of sprouting was calculated, and then it was predicted whether they could be detected based on the volume of the water subsample that was tested.

Results: The safety of the sprouts after 48 h of sprouting was a result of two factors: (1) detection of pathogens by testing of spent irrigation water; and (2) removal of the pathogens by repeated wash cycles. These factors are diametrically opposed and create a middle ground which made it difficult to ensure the safety of the sprouts. The middle ground was defined by cases where washing reduced organism levels to less than the number required to be detected by testing of spent irrigation water (estimated to be >2.5 log CFU based on weight of sprouted seed, volume of sprout irrigation water and portion removed for testing) but did not eliminate them, thus leaving residual organisms in the sprouts.

Significance: The middle ground could not be easily reduced by variations in wash cycle frequency. The results suggest that microbial testing of spent sprout water does not ensure that sprouts are free of contamination to the degree that is commonly assumed.

P1-129 Assessment of Risk of Salmonellosis from Consumption of Pistachios

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Introduction: It has recently been recognized that there is a risk of contracting Salmonellosis from consumption of roasted in-shell and kernel pistachio products.

Purpose: The purpose was to characterize the risk category of raw pistachios, and determine what degree of intervention, if any, would be necessary to provide an appropriate level of protection for public health.

Methods: A Monte Carlo approach was used to assess the risk of Salmonellosis from the consumption of pistachios. Starting conditions were based on prevalence rates observed during hold and release testing of incoming pre-processing in-shell and kernel materials, and population levels of *Salmonella* enumerated in test lots for which confirmed positive results were obtained.

Results: The assessment found that without microbial reduction due to roasting, in-shell pistachios would be regarded as a moderate risk category food item using a very conservative risk scale based on the number of hospitalization cases per billion servings basis. Application of an intervention with a reductive capacity of 3 logs or more was found to be sufficient to ensure that there would be a 95% probability that less than 1 hospitalization per year would occur for in-shell product, corresponding to a very low risk category according to the risk ranking scheme used in this risk assessment. Pistachio kernels, representing only about 10% of the market, would be considered a high risk item and a reductive capacity of 4 logs would be necessary to achieve the same risk category.

Significance: The FDA has recently released a proposed "Guidance for Industry: Measures to Address the Risk for Contamination by *Salmonella* Species in Food Containing a Pistachio-Derived Product as an Ingredient" which recommends use of a validated intervention process capable of delivering a 5-log reduction. This risk assessment found that, while control is clearly needed, the public health goal of producing very low risk pistachio products can be met by using less stringent thresholds of performance for intervention processes.

P1-130 Microbial Profiling of Pistachio Processing Using Indicator Organisms

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Introduction: During pistachio processing a number of operations take place which could affect the microbial profile of pistachios. Few data are available on the impact of these operations.

Purpose: This study evaluated the levels of indicator organisms at multiple points in the pistachio production process in order to acquire data which would allow pistachio processors to more accurately estimate the impact of antimicrobial interventions as a function of where they are placed in the process flow.

Methods: Samples were collected at multiple points in the production process at three pistachio production facilities during two sampling sessions in September, 2009, and March, 2010. A total of eight key locations were identified along the production process. Considering subdivisions of the process flow (for example, separation of 'floaters' and 'sinkers' during an initial wash and color sorting into stained and acceptable) samples were collected at a total of fourteen points. All samples were analyzed for the indicator organisms aerobic plate counts (APC), total coliforms (TCC) and generic *E. coli* (ECC).

Results: Initial levels for indicator organisms were high on freshly hulled pistachios and after an initial wash. APC, TCC and ECC levels of approximately 8, 7 and 3 log CFU/g were observed. After drying and prior to storage in silos, levels of 'sinkers' dropped substantially (to 2.8, 1.1 and 0 log CFU/g, respectively). Levels remained fairly constant until the last step of roasting, which lead to final levels of approximately 1.1, 0.1 and 0.0 log CFU/g, respectively. Floaters, which are used to produce kernel pistachio products, exhibited a similar pattern except that they generally had higher and more variable levels of the indicator organisms.

Significance: The results indicated that the early step of drying had the biggest impact on microbial burdens, and suggested that floaters require application of more stringent interventions to reduce microbial loads.

P1-131 Microbiological Quality of Buffet and Take-out Rice Dishes in Florida

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Introduction: Cooked rice from Asian restaurants and take-out stores has been associated with *Bacillus cereus* food poisoning. Such illnesses are caused by improper cooking, cooling or storage of rice, resulting in germination and growth of *B. cereus* spores.

Purpose: We investigated how the microbiological quality (total aerobic plate count [APC], *E. coli* / coliform, *Staphylococcus aureus*, and *B. cereus*) of cooked rice from Asian restaurants and take-out stores was affected by temperature and pH, seasonality, style of cuisine, preparation method (steamed versus fried) and setting (buffet tables versus point-of-sale preparation).

Methods: Samples (n = 100) were purchased at point-of-sale and/or from buffet tables, transported to the lab without refrigeration to simulate consumer practices, and evaluated for temperature (at point-of-sale) and pH. Populations of *B. cereus*, APC, *E. coli* / coliform, and *S. aureus* were analyzed using the Food and Drug Administration's Bacteriological Analytical Manual (FDA-BAM) 3-tube most probable number (MPN) series, 3M Petrifilm™ aerobic count plates, *E. coli* / coliform count plates, and Staph Express count plates, respectively.

Results: 1.0% of samples tested positive for coliform (10^2 to $<10^3$ CFU/g). 1.0%, 1.0%, and 6.0% contained $<10^2$ CFU/g of *E. coli*, *S. aureus*, and *B. cereus*, respectively. *E. coli*, coliform, and *S. aureus* were detected in fried rice from buffets. Higher *B. cereus* counts and lower temperatures ($P < 0.0001$) were found in buffet samples than in samples of point-of-sale steamed rice kept warm in rice cookers or freshly prepared fried rice.

Significance: Although the majority of rice samples were of acceptable microbiological quality, the presence of *E. coli* / coliform and *S. aureus* in buffet rice samples indicates post-cooking contamination by consumers and/or food handlers, suggesting compromised personal hygiene standards and/or sanitation practices.

P1-132 Growth of *Vibrio parahaemolyticus, Salmonella* sp. and *Staphylococcus aureus* during Short-term Temperature Abuse of Raw Shrimp

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Introduction: Vibrio parahaemolyticus (VP), *Salmonella* spp. (SAL), and *Staphylococcus aureus* (SA) may be present or introduced into shrimp during processing and handling. Foodborne illnesses caused by these pathogens can result from consuming raw, undercooked, or re-contaminated shrimp. Seafood processors must also prevent the growth of these pathogens that may occur during short-term temperature abuse in processing and transportation. Kinetics data are needed to guide the decision making process and control hazards.

Purpose: This study determines the Lag-Phase Duration (LPD) and Growth Rate (GR) values of VP, SAL and SA in shrimp incubated isothermally over a range of temperatures representative of temperature abuse conditions. The kinetics data will be used as part of the interval-accumulation predictive tool, Time-Temperature Pathogen Predictor (T2P2).

Methods: Three separate cocktails of inocula were prepared by combining five select strains grown overnight at 35°C for each organism. Raw, shell-less, macerated shrimp (25.0 g) was inoculated with each cocktail (10^4/g) and incubated at 2.8°C increments over 10 – 43.3°C. Samples were withdrawn at timed intervals, diluted and plated on appropriate media, then incubated at 35°C for 18 – 48 hours. Each isothermal experiment was replicated three times. Kinetics data were fitted with DMFit to obtain LPD and GR values.

Results: For all organisms, LPD decreased and GR increased as temperature increased from 26.7 – 37.8°C. For example, LPD for SAL decreased from 104.8 to 95 to undetectable (min) whereas GR for SA increased from 0.004 to 0.014 to 0.018 {log (CFU/mL)/min} at 26.7, 32.2 and 37.8°C, respectively. Comparatively, the respective GR of VP, SAL and SA at 26.7°C was 0.02, 0.005 and 0.004 {log (CFU/mL)/min}, indicating differences in GR among the organisms.

Significance: The kinetics data provided here expands the capabilities of T2P2 to include specific pathogen behavior in shrimp when exposed to various temperatures, and further supports risk mitigation strategies for determining critical limits (HACCP) and decision making for process deviations.

P1-133 Growth Comparison of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in Broth and Seafood

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Introduction: The isolation of *Vibrio* species from various seafood is not unusual, and thus seafood consumption may cause health risks for consumers. Predictive modeling has been used for exposure assessment in microbial risk assessment. However, the development of growth predictive modeling of *Vibrio* spp. in seafood has been rarely studied.

Purpose: The objective of this study was to investigate growth kinetics of *V. vulnificus* and *V. parahaemolyticus* inoculated in broth and sashimi and to conduct risk assessment of seafood consumption by comparison of growth data of *V. vulnificus* isolated from oysters in the market.

Methods: For broth model, *V. parahaemolyticus* (ATCC 33844) and *V. vulnificus* (ATCC 27562) were inoculated into broth and stored at 11, 13, 18, 24, 30 and 36°C. Ten g of salmon and flatfish sashimi were inoculated with *V. parahaemolyticus*, respectively. Twenty-five g of raw oysters purchased from the market were stored at 18, 24, 30 and 36°C to investigate the growth kinetics of *Vibrio vulnificus* isolate in oysters. Growth kinetic parameters including lag time (LT), specific growth rate (SGR), and maximum population density (MPD) were determined at each temperature by the Gompertz equation.

Results: There were no significant differences in growth kinetics between *V. parahaemolyticus* and *V. vulnificus* in broth, regardless of temperature. However minimum growth temperature for *V. vulnificus* and *V. parahaemolyticus* was 11°C and 13°C, respectively. When comparing salmon to flatfish, flatfish showed a faster growth rate than salmon. According to the result of biochemical identification of isolates, only *V. vulnificus* was confirmed with the range of 1 – 1.3 log CFU per g of oyster. The maximum population density of *V. vulnificus* isolate in oysters was 4 log CFU/g after 3 days of storage, regardless of temperature.

Significance: Shelf life of sashimi in the market should be considered according to fish species. Since the infectious dose of *V. vulnificus* for the high risk group is 10^2 CFU/g, careful storage and consumption guidelines for oysters must be emphasized for consumers in the retail market.

P1-134 Isolation of *Salmonella* spp. from Surface Waters in Florida over a Five-month Period

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Introduction: Outbreaks of salmonellosis have been associated with the consumption of contaminated fresh produce. A potential source of contamination is surface water that is in, or around, produce fields.

Purpose: Our objective was to screen surface water samples monthly from Florida for the presence and concentration of *Salmonella* spp. and potential indicator microbial and chemical characteristics.

Methods: Ten-liter samples were taken monthly for 5 months from 6 locations in each of three counties. At time of sampling water temperature, pH, turbidity and conductivity were measured. Air temperature was also recorded. Samples were concentrated using tangential flow filtration after overnight storage at 4°C, enriched overnight at 37°C in double strength lactose broth, and further concentrated, using immunomagnetic separation. DNA was extracted from the immunomagnetic beads and real-time PCR was performed. A retain from positive samples was carried through a *Salmonella* MPN. *Salmonella* isolates were confirmed using PCR for the presence of *invA* and/or *oriC* genes. Aerobic plate counts and coliform/*E. coli* MPN, were also done.

Results: All samples were positive for *Salmonella* following tangential flow filtration – real-time PCR. Significant differences ($P < 0.05$) exist between *Salmonella* MPN levels for the three counties sampled. Only one county showed a significant difference ($P < 0.05$) in *Salmonella* MPN levels between sampling months. The strongest correlations between *Salmonella* concentration and other parameters were air temperature and *E. coli* MPN, which were found to have correlation coefficients of 0.30 and 0.26, respectively. When looking at just one county, a correlation coefficient of 0.43 was found for the air temperature. All other measured attributes showed correlation coefficients between 0.18 and -0.15.

Significance: This work elucidates the continued presence of *Salmonella* in Florida surface waters, not strongly correlated to the standard coliform/*E. coli* testing commonly used to evaluate produce water safety.

P1-135 A Framework for Validation of the Microbial Safety of Cooked Chilled Foods

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Introduction: Cooked chilled foods, more aptly known as REPFEDs (refrigerated and processed foods of extended durability), are a very heterogeneous group of food products. To assure the microbial safety of these products, the food industry relies on a combination of mild heat treatment (i.e., pasteurization), refrigerated storage and consumer instructions for handling and preparation.

Purpose: This research assessed the potential risk to the consumer for different types of REPFEDs, based on the production process, labeling and data collection on prevalence and survival of pathogens. It provides a framework for the validation of the microbial food safety of REPFEDs.

Methods: The production-processes of 5 Belgian REPFED-producing companies were analyzed according to their microbial risk profile. To gain insight into the current food safety situation, both historic (n =1,533) and new (n = 90) analysis results for 3 pathogens (*B. cereus, L. monocytogenes* and sulphite-reducing *Clostridia*) were collected for final products on the day of production and for final products at the end of shelf life. The pasteurization value (*P*-value) for heat treatment at the consumer phase was determined for 50 products by simulating the proposed heat treatment as recommended on the label. Finally, one high-risk product (paella) was challenge-tested (in 12-fold) for survival of *L. monocytogenes* during heat treatment at the consumer phase.

Results: Three types of cooked chilled foods could be distinguished based on the heat treatment applied during the production process (P90 =10, P0 = 2 or no safe harbor), while 4 types of REPFEDs could be distinguished based on the heat treatment applied at consumer phase (Ready-to-eat, ready-to-reheat, ready-to-heat and ready-to-cook). The combination of both heat treatments (production process and consumer phase) ultimately determines the risk of the REPFED to the consumer. None of the analyzed products (n =1,533) carried unacceptable numbers for any of the three pathogens. Of the 50 products that were reheated only 20% obtained a *P*-value sufficiently high to eliminate *L. monocytogenes* (P0=2). During the challenge tests of *L. monocytogenes* in paella, the pathogen was able to grow in all 12 replications and remained present in 7 out of 12 replications after reheating at the consumer level.

Significance: Results indicate that the current microbial safety of REPFEDs is good, but that a thorough validation of both production-process and final product is necessary to guarantee the food safety. If reheating by the consumer is necessary for food safety, then this process should also be validated.

P1-136 Evaluating Food Safety Management Performance in a Food Service Establishment According to a Microbiological Assessment Scheme

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Introduction: Poor food safety management systems (FSMS), and an attendant increase of food safety risks in professional kitchens, may impact a significant number of consumers. A national food consumption survey in Belgium demonstrated that more than 35 percent of the population consumed more than 25 percent of their daily energy intake out of home.

Purpose: A Microbial Assessment Scheme (MAS) was adapted in a food service establishment, vertical through the production process, from raw materials to final products, in order to measure the microbiological performance of the FSMS.

Methods: MAS supports deciding where and how to take a sample, at what frequency, how to conduct microbial analyses, how to interpret results and judge the outcome in perspective of the FSMS. MAS was applied on three different production processes, namely the preparation of a sandwich/salad, the production of a hot meal starting with unprocessed raw or undercooked material and preparation out of packaging, the production of a hot meal starting with cooked products and regeneration in pack, all in the same food service establishment.

Results: The results of total viable aerobic count were not in accordance with microbiological guidelines on food contact surfaces and gloves. However all hygiene parameters, namely *E. coli*, *Enterobacteriaceae* and *S. aureus*, were according to the legal criteria and or guidelines. Also, no important pathogens like *Salmonella* spp. and *L. monocytogenes*, were detected.

Significance: In general, it can be concluded that the catering establishment has a well-functioning FSMS for their main production processes. The application of MAS in a catering setting can help to analyze the performance of a FSMS and to assign points of attention for a better performing FSMS.

P1-137 Restaurant Food-cooling Practices

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Introduction: Improper cooling of hot food is a significant source of foodborne illness and should be the focus of intervention efforts. As half of all foodborne illness outbreaks are associated with restaurants, it is particularly important to focus on restaurant cooling practices. However, effective interventions require knowledge of current policies and practices, and currently, little data exist on restaurant cooling policies and practices.

Purpose: The purpose of this study was to collect descriptive data on these topics. This study was conducted by the Environmental Health Specialists Network (EHS-Net), a collaborative forum of federal, state, and local environmental health specialists and epidemiologists working to better understand the environmental causes of foodborne illness.

Methods: EHS-Net personnel collected data in 420 randomly selected restaurants through interviews with restaurant managers and observations of food-cooling practices.

Results: Improper cooling practices were not uncommon. Cooling food times or temperatures were not monitored during cooling processes in 41% of restaurants. In 33% of observations in which an ice bath was used to cool food, ice and water were not filled to the top of the cooling food. In 39% of observations in which food was cooled through refrigeration, the food depth was not shallow, in 34% of these observations the cooling food was not ventilated, and in 24% of these observations open air space was not provided around the food cooling containers.

Significance: These data indicate that many restaurants engage in improper cooling practices. Interventions are needed to improve these practices, and the findings from this study can help target those interventions.

P1-138 Applying Process-based Analytics to Audit Results for Process Management and Improvement

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Introduction: Third-party and internal audits are basic tools in demonstrating Food Safety Management System (FSMS) efficacy under programs such as GFSI. This study examines how audit results can be successfully used for process management in addition to verifying the FSMS.

Purpose: The general practice among food processors is to use audit results only for FSMS certification and not for process management and improvement. This paper presents a strategy to apply process-based risk assessment and analytics to audit results to both enable efficient management by exception and to improve FSMS performance.

Methods: Prerequisite programs (PRPs) are examined from a management perspective using the ISO 22000 model for verification and management review. Analytical methods such as Pareto analysis and control charting are applied to assess the effectiveness of plant sanitation process. This allows the development of strategies to effectively reduce risk, maintain and improve the PRP, and reduce the incidence of PRP failures.

Results: Methods such as control charting alert management when PRP performance is deteriorating and when critical limits are exceeded. Plant sanitation APC results are used as the case study.

Significance: Applying process analytics to PRP audit data enables food processors to manage the operation more effectively rapidly. This reduces the likelihood of food safety failures. By applying modern Enterprise Manufacturing Intelligence (EMI) methods food processors can deliver better and more dependable food safety outcomes.

P1-139 Estimation of Food Commodity Intakes from the Korea National Health and Nutrition Examination Survey

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Introduction: Estimation of food commodity intakes is important to control the potential risks of hazardous substance in foods which are contaminated intentionally or unintentionally in food commodities. The Korea diet databases provided by one-day recall survey in Korea National Health and Nutrition Examination Survey (KNHANES) are good resources to estimate intakes of various food commodities. However, there are limitations in use of the KNHANES diet databases which contain food ingredients and recipes but are not formatted as food commodities. Therefore, the databases cannot be directly used for the estimation of toxicity exposure based on food commodity intake.

Purpose: Herein, software to calculate food commodity intakes from the KNHANES diet databases has been developed.

Methods: Algorithms adopting reasonable calculation strategies are developed to convert food ingredients and consumptions of the KNHANES diet to the food commodity intake.

Results: The software can calculate the total of people who consumed foods: men, women; age under 20, equal to or over 20 and percentages between consuming people only and participants in the KNHANES diet statistically. The average daily food commodity intakes, for example, gender, age, people at 5% upper intake limit and etc., are calculated using those algorithms with 95% confidence interval. This software has been designed to update periodically by adding the latest KNHANES diet databases and a variety of recipes.

Significance: This software would contribute to estimate human exposure to toxic materials such as residual pesticides and corresponding their potential risks.

P1-140 Changes of Antimicrobial Resistant and Pathogenic Bacteria along the Pork Processing Chain

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Introduction: The spread of antimicrobial resistant bacteria in food animals is of great concern worldwide because antimicrobial resistant, and pathogenic bacteria are able to contaminate the pork and to be transferred to the consumer during the processing from slaughterhouses to retail shops.

Purpose: The changes of pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus* spp., from pork collected along the pork processing chain from slaughterhouses to retail shops were investigated with the qualitative and quantitative detection and the profiles of antimicrobial resistant.

Methods: From March to July 2010, a total of 598 swab samples were collected from pre-chill and post-chill carcasses (n=313) in 5 slaughterhouses, from post-cutting pork (n=179) in 4 processing plants and from retail pork meats (n=107) in 4 retail shops. The swabs were applied to quantitative real-time PCR (qRTi-PCR) with SYBR green and species-specific primers for *E. coli* , *S. aureus* and *Enterococcus* spp. At the same time, bacteria were isolated according to the Korean Food Standards Codex (2009) and antimicrobial resistant profiles were analyzed by disk diffusion test according to the guidelines of the Clinical Laboratory Standard Institute (CLSI).

Results: Quantitatively, the detection rates and the bacterial cell counts of *E. coli* and *S. aureus* among the samples were decreased along the processing. In *Enterococcus* spp., on the other hand, the bacterial cell counts were not changed along the processing chain, while the detection rates were decreased as other bacterial spp. Generally, *E. coli* strains were highly resistant against beta-lactams and tetracycline. *S. aureus* strains were more resistant to penicillins, tetracycline and erythromycin than to other antibiotics and *Enterococcus* spp. strains were more resistant to tetracycline, erythromycin and ciprofloxacin than to others. The detection rates of antimicrobial-resistant *E. coli* and *S. aureus* were decreased while that of *Enterococcus* spp. was not during processing. On the other hand, the multidrug resistance rates of the *E. coli* were not changed while those of *S. aureus* and *Enterococcus* spp. were decreased during processing.

Significance: Generally, the contamination of pork by pathogenic bacteria tended to decrease quantitatively. The antimicrobial resistance showed diverse patterns depending on the bacteria. This study provides scientific data upon which to base risk assessments for the effective control of pathogenic and antimicrobial-resistant bacteria in the pork processing chain from slaughterhouses to retail shops.

P1-141 Performance of a New Molecular Platform for the Detection of *Listeria monocytogenes* and *Listeria* species

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Introduction: Testing for *Listeria monocytogenes* and *Listeria* species is a critical component of food safety programs, but current methods are perceived to be complicated, lengthy or expensive. To address the need for a less complex, more rugged detection method, a new molecular detection platform was designed.

Purpose: To evaluate the performance of new *L. monocytogenes* and *Listeria* species detection methods for 1) false negative rates; 2) false positive rates; and 3) fractional recovery in comparison to cultural and PCR methods.

Methods: Inclusivity and exclusivity studies were performed using > 80 different *Listeria* and non-*Listeria* cultures. Organisms were cultured according to the defined method and then diluted in fresh enrichment broth prior to replicate testing using the new molecular methods. Non-Listeria that did not multiply adequately using the enrichment were propagated in tryptic soy broth. True positive and true negative states were determined by each organism's genetic or biochemical identity. In addition, method comparison and fractional recovery studies were performed by testing 20 replicates of ten different enriched sample matrices that had been artificially contaminated at a low level and then acclimated for several days. In addition, five non-inoculated portions were tested using each method. Split matrix samples were also evaluated using the ISO 11290-1 method and a commercial PCR method. A Chi-square test was used to compare the methods for significant differences.

Results: Inclusivity and exclusivity rates of 100% were determined using pure cultures. Significant differences were identified for several matrices (three each for *L. monocytogenes* and *Listeria* species) evaluated using the chi-square statistic. Presumptive positives were verified by streaking to MOX agar and/or by qPCR.

Significance: A new detection system was evaluated using cultures of known identity and in comparison to cultural and contaminated matrices in comparison to cultural and PCR methods. The new methods were found to be reliable and accurate and to offer advantages to the end user, including a quicker time to result compared to the cultural method and a smaller, more rugged instrument and less complex sample preparation compared to the PCR method.

P1-142 Performance of a New Molecular Platform for the Detection of *Salmonella* and *Escherichia coli* O157

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Introduction: Testing for *Salmonella* and *E. coli* O157 is a critical component of food safety programs, but current methods are perceived to be complicated, lengthy or expensive. To address the need for a less complex, more rugged detection method, a new molecular platform was designed.

Purpose: To evaluate the performance of new *Salmonella* and *E. coli* O157detection methods for 1) false negative rates; 2) false positive rates; and 3) fractional recovery in comparison to cultural and PCR methods.

Methods: Inclusivity and exclusivity studies were performed using > 200 different cultures (for *Salmonella*) and > 80 different cultures (for *E. coli* O157). Organisms were cultured according to the defined method and then diluted in fresh enrichment broth prior to replicate testing. True positive and true negative states were determined by each organism's genetic or biochemical identity. In addition, method comparison and fractional recovery studies were performed by testing 20 replicates of twelve different enriched sample matrices (eight for *Salmonella* and four for *E. coli* O157) that had been artificially contaminated at a low level and then acclimated for several days. In addition, two or more non-inoculated portions were tested using each method. Split matrix samples were also evaluated using the ISO 6579 or ISO 16654 methods and a commercial PCR method. A Chi-square test was used to compare the methods for significant differences.

Results: Inclusivity and exclusivity rates of > 99% were determined using pure cultures. No significant differences were identified for the twelve matrices evaluated using the Chi-square statistic.

Significance: A new molecular platform was evaluated using cultures of known identity, and artificially contaminated matrices were tested in comparison to cultural and PCR methods. The new methods were determined to be reliable and accurate and to offer substantial advantages to the end user, including a quicker time to result compared to the cultural method and a smaller, more rugged instrument and less complex sample preparation compared to the PCR method.

P1-143 Comparison of Methods for Enumeration of Yeast and Mold in Butter

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Introduction: Methods to measure the microbiological quality of butter must be designed to account for its composition and physical structure. Various tests can be used to determine the quality and safety of butter or to determine sources of contamination during the manufacturing process. The microflora of butter reflects the quality of the cream, the sanitary conditions of the equipment, and the environmental and sanitary conditions during packaging and handling. The development of yeasts and molds on the surface of butter can cause surface discoloration and flavor problems.

Purpose: The objective of this study was to compare cultural methods for enumerating yeasts and molds in naturally contaminated butter.

Methods: The samples were simultaneously analyzed using four methods: Petrifilm YM plate, DRBC agar, acidified PDA agar, and Sabouraud agar, plating 1 mL from 1:10 and 1:100 dilutions, in duplicate, and incubated at 22°C/5 days.

Results: The results were compared, submitted to statistical analysis using Kruskal-Wallis test (Minitab® Release 14.20 Statistical Software) and Linear Regression. For statistical purposes, the results were transformed in Log using the equation "1+ $\log_{10}$UFC". Results showed that counts between Petrifilm YM plate and traditional methods were not statistically different. The R^2 was 98.4%, 99.9% and 98.6% for the comparison between Petrifilm YM and DRBC agar, acidified PDA agar and Sabouraud agar, respectively. The p-value was > 0.05 for all combinations.

Significance: This study demonstrates 3M Petrifilm YM plates can be considered an alternative method to the traditional enumeration of yeast and mold in butter. Petrifilm plates can decrease data variability and improve lab productivity by eliminating medium preparation and material sterilization.

P1-144 Development of a Rapid Protocol for Enumerating Coliforms in Yogurt

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Introduction: Yogurt normally has a low pH that generally prevents the growth of most spoilage and pathogenic organisms. Microorganisms that cause deterioration of fermented milk products can enter products through poor sanitation techniques or can be introduced by the addition of flavoring materials such as fruit.

Purpose: Coliform counts may be used as indicators of adequacy of processing sanitation but, if present, decline rapidly after manufacture of yogurt, so this study was carried out to find out the best protocol to enumerate coliforms in less than 17 hours using the 3M™ Petrifilm™ Rapid Coliform Count Plate (RCC).

Methods: The samples were spiked with generic *E. coli* incubated overnight at 35°C in TSB broth, in order to inoculate 1 mL of TSB broth per each 100 g of product from dilution 10 fold 5. Several combinations were evaluated using four diluents (BPW pH 7.0, BPW pH 9.0, Modified BPW and M broth), four dilutions (undiluted, 1:2, 1:5 and 1:10) and two periods of incubation (12 and 17 hours). All of them were submitted to a recovery step, by keeping them one hour at room temperature after dilution, then 1 mL was plated onto Petrifilm RCC plate in duplicate and incubated at 35°C.

Results: The amount of 290 results were submitted to Main Effects Plot and the best combination showed was BPW pH 7.0, diluted 1:5 or 1:10, one hour recovery step, plated onto Petrifilm RCC plate and incubated at 35°C/17 hours. To get the best period of incubation, this combination was evaluated at 10, 11, 12, 13 and 17 hours of incubation compared to VRB agar incubated at 30°C/24 hours plated directly.

Significance: The amount of 294 results were analyzed by Pearson Correlation and showed that Petrifilm RCC plate in 12 hours, diluted 1:5 is an excellent choice compared to VRB agar, by recovering more coliforms in yogurt and helping the dairy industry obtains reliable results more quickly.

P1-145 Rapid High-throughput Microtitre Plate-based Analysis of Bacterial Load (TVCs)

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Introduction: A rapid, high-throughput method for the assessment of microbial metabolism is presented and applied to the determination of Total Viable Counts in a variety of food types.

Purpose: A rapid, high-throughput method for the assessment of microbial metabolism is presented and applied to the determination of Total Viable Counts in a variety of food types.

Methods: The method uses a water-soluble oxygen-sensitive phosphorescent probe (GreenLight™ 960 probe) to monitor the oxygen consumption of microbial samples. A proof of concept study was carried out to identify the appropriate threshold (probe signal) at which a marked increase in sample oxygen consumption is observed. The higher the initial microbial load, the earlier this threshold level is reached. To determine the utility of this metric in the assessment of microbial contamination in food samples, a comparison study was run with the industry standard 'aerobic plate count' method (ISO:4833:2003), and a strong correlation was observed($r^2 > 0.90$).

Results: The developed method is shown to be a viable alternative to conventional culture methods allowing rapid, high-throughput determination of TVC (30°C) in meat samples. For example, in beef samples, contamination levels as low as 1 x10^3 CFU/g can be measured within 12 hours instead of the 72 hours required by the conventional method, while samples at ~1 x10^8 CFU/g are identified within an hour. Speed to a CFU/g result is therefore vastly improved. The assay is also less labor and material intensive. Data on microbial contamination in raw milk is also presented.

Significance: The presented rapid TVC test provides a simple, fast, convenient and high throughput alternative to conventional TVC testing. Also, as GreenLight™ 960 allows for the specific detection of microbial oxygen consumption, such measurements can also provide insight into the metabolic effect of various manipulations.

P1-146 Rapid Strain-to-strain Identification of *Lactobacillus* and *Bacillus* Wild Isolates Using MALDI TOFMS

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Introduction: Besides DNA sequencing, chemical analyses of microbial cell components such as MALDI (Matrix Assisted Laser Desorption/Ionization) TOFMS (Time of Flight Mass Spectrometry) have greater advantages in rapid species identification of colonies isolated on agar plates. This approach deserves further attention in instant strain-to-strain recognition of wild isolates from environmental analysis and process inspections.

Purpose: We have improved the species identification procedures of MALDI TOFMS analysis so that individual isolates of *Lactobacillus plantarum* or *Bacillus cereus* are further distinguished from each other within the species, respectively. It enables us to achieve strain-specific identification of wild isolates within 60 minutes.

Methods: Three wild isolates in *Lactobacillus plantarum* and *Bacillus cereus* were applied in this study, and approximately 5–10 mg of bacterial cells were suspended in water for preparation. Ribosomal proteins were targeted, as they were diversified greatly within closely related species. Since the analytical resolution was primarily restricted by insufficient signal recovery and poor reproducibility in spectrum pattern analysis, our efforts were focused on the improvement of ribosomal protein detection with an assist of pattern analysis software. A mass spectrometer, Ultraflex III MALDI TOF/TOFMS (Bruker Daltonics) was used in this study.

Results: Protein recovery was improved by a serial treatment with ethanol, formic acid and acetonitrile with a Vortex mixer. Two µl of supernatant were air dried on the MALDI plate and the use of one µl of matrix reagent overlay (sinapinic acid) was found best suited in protein detection. In both sets of three strains, *Lactobacillus plantarum* and *Bacillus cereus*, each strain exhibited a distinctive spectrum. When culture conditions were reproduced, the characteristic spectrum of each strain was retained identical.

Significance: The time required for an assay was 30 minutes for extraction, 20 minutes for plate application and 10 minutes for MALDI TOFMS analysis. Rapid strain-to-strain identification tools were successfully demonstrated in important microorganisms in food processing and food safety.

P1-147 Comparison of Agar Media Types and Plating Techniques in the Recovery of Yeasts and Molds from Apple Juice

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Introduction: Yeast and mold contamination of food and beverage products can occur during processing and handling and can have adverse effects on safety and quality. Different types of microbiological media are available commercially to select for yeasts and molds.

Purpose: The purpose of this study was to compare different agar media types and plating techniques to maximize the recovery of yeast and mold from apple juice.

Methods: Design of experiments was conducted for both yeasts and molds. The randomized design compromised of four factors (yeast or mold strains, inoculum levels, agar types and plating techniques) at multiple levels. The yeast strains tested were *Yarrowia lipolytica* (a dairy isolate), *Candida lusitaniae* (a brewery isolate), and *Schizosaccharomyces pombe* (a food isolate), while the mold strains were *Aspergillus niger* ATCC 16404, an unknown dairy-field isolate, and an unknown beverage-field isolate. Pasteurized apple juice was inoculated with a high (10^5 to 10^6 CFU/ml) or low (10^2 to 10^3 CFU/ml) inoculum level of each individual yeast or mold strain. Samples were diluted when necessary and survivors were plated on five different agar media types via spread plate or pour plate technique. The agar media selected for this study included malt extract agar (MEA), potato dextrose agar (PDA), oxytetracycline glucose yeast extract agar (OGYE), Sabouraud dextrose agar (Sab) and dichloran rose Bengal chloramphenicol agar (DRBC). Plates were incubated up to 5 days at 26°C and counts were enumerated at Day 3 and Day 5.

Results: No significant differences ($P > 0.05$) were observed between spread and pour plate techniques for both yeast and mold. The type of yeast or mold strains as well as inoculum levels showed significance in the recovery ($P < 0.05$). There was no difference in the media type used for recovering yeast ($P > 0.05$) but there was a difference for mold ($P < 0.05$). DRBC contained the spreading of the mold, hence, was easier to enumerate. Visual observations indicated larger colonies on Day 5 compared to Day 3 but no increase in numbers.

Significance: Certain agar media may be more suitable to isolate and recover molds from food and beverage products.

P1-148 Selective Detection of Viable Spoilage *Pseudomonas* spp. Using Propidium Monoazide-coupled TaqMan Real-time PCR

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Introduction: Pseudomonas spp. strains are abundant in the environment, various foods and hosts as commensal bacteria or opportunistic pathogens, and psychrotrophic *Pseudomonas* spp. is a common spoilage agent in pasteurized milk. Rapid and specific detection of viable *Pseudomonas* spp. cells is preferred for proper dairy quality control. However, conventional culturing detection is labor intensive and time consuming. On the other hand, DNA molecules from dead cells can cause a false-positive result by DNA amplification based rapid detection assays. Propidium monoazide (PMA) can penetrate damaged cell membranes and form crosslinkage with DNA molecules resulting in inhibition of amplification. Several studies have reported its application in conjunction with PCR, to reduce false-positive results by heat inactivated cells. However, there is a lack of understanding of its applicability in assessing viable populations after treated with other microbial inactivation methods commonly used in the food environment.

Purpose: The purpose of this study was to evaluate the efficacy of a PMA-coupled TaqMan real-time PCR assay in selective detection of viable *Pseudomonas* spp. cells from dead ones exposed to several bacteria inactivation treatments.

Methods: TaqMan real-time PCR primers and probe sets targeting conserved regions of the 16S rRNA gene and Ornithine decarboxylase (ODC) gene were designed. The specificity of the real-time PCR assays was examined using representative microorganisms commonly found in the dairy environment. After heat, acid and disinfectant inactivation, *Pseudomonas* cells were treated with PMA, followed by DNA extraction and real-time PCR assessment.

Results: Using the 16S rRNA and ODC-based primers and probe sets, specific detection of *Pseudomonas* spp. by PMA-coupled TaqMan real-time PCR detection platforms was achieved without cross-activity to other bacteria commonly found in the dairy environment. PMA treatment successfully minimized false-positive amplification signals by dead cells from different inactivation treatments.

Significance: The results suggested that the established PMA-coupled TaqMan real-time assays can be used for rapid and specific detection of viable spoilage *Pseudomonas* spp. cells. Application of the developed detection system can enhance quality control and minimize spoilage incidence in processed foods.

P1-149 Variations in the Detection of Staphylococcal Enterotoxin B is Compromised in Thermally-processed Foods

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Introduction: Staphylococcal food poisoning in man has been reported after ingestion of thermally-processed foods, and *Staphylococcal enterotoxin B* (SEB) is known to display a relative resistance to thermal inactivation. Toxin detection using antibody-based assays in thermally-processed foods is compromised, leading to false negatives in contaminated samples.

Purpose: Development of new approaches for the detection of thermally-processed SEB within food matrices.

Methods: SEB or PBS was introduced into food matrices including hamburger meat, chicken nuggets, egg substitute and spaghetti sauce. The concentrations of SEB (50 µg/ml, 5 µg/ml, 500 ng/ml, and 5 ng/ml) used in our assays are concentrations capable of inducing human disease. Cooked and uncooked food matrices were weighed, incubated with PBS at room temperature for two hours, and centrifuged (20 minutes x 1500 g) and 150 µl aliquots of each sample was applied to lateral flow devices (LFDs). Raw and cooked hamburger supernatants were further subjected to molecular weight filtration using Ultricon filters and the filtrate assayed using the LFD.

Results: SEB was easily detected in supernatants derived from all uncooked foods. Cooked foods presented variable results; toxin was easily detected in cooked spaghetti sauce, but not in cooked eggs. SEB was found to be sequestered within distinct pockets of cooked hamburgers. Molecular filtration of supernatants from the raw and cooked meat patties spiked with 1.1 µg per gram of meat showed that SEB was present in molecular weight fractions of 50kDa and 100kDa.

Significance: The toxin was sequestered in high molecular weight complexes in the cooked food, making it unavailable for detection with antibodies in ELISA based kits. Detection of the toxin may be easier when the complexes are isolated, thus providing an approach for the examination of thermally processed foods that are contaminated with SEB.

P1-150 Evaluation of TA10 Broth for Recovery of Heat- and Freeze-injured *Salmonella* from Beef

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Introduction: Salmonella is one of the most commonly reported causative agents of bacterial foodborne illness worldwide, and the availability of sensitive methods for detection of *Salmonella* is critical. An effective pre-enrichment medium is necessary for detecting *Salmonella* by both conventional culture and molecular detection methods.

Purpose: We optimized the components of pre-enrichment TA10 broth developed for simultaneous growth of *Salmonella* spp., *Listeria monocytogenes,* and *Escherichia coli* O157:H7. In this study, we evaluated TA10 broth for recovery of heat- and freeze-injured *Salmonella* in beef by conventional culture protocols.

Methods: The Bacteriological Analytical Manual (BAM) *Salmonella* pre-enrichment broth (lactose [LAC] broth), buffered peptone water (BPW) and universal preenrichment (UP) broth were compared with TA10 broth, developed in our laboratory, for recovery of heat- and freeze-injured *Salmonella* (55°C for 2-20 min and -20°C for 2 months, respectively) from beef. Beef samples were contaminated with single *Salmonella* serovars, and contamination levels of 0.44 to <0.001 MPN / g and 0.74 to 0.14 MPN / g were used for heat- and freezing-induced injury studies, respectively. Twenty test portions (25 g) of the contaminated beef were pre-enriched in each broth, and the BAM *Salmonella* culture method was used thereafter.

Results: There was a significant difference in recovery of heat-injured *Salmonella* using TA10 broth compared to LAC broth (189 versus 156 positive samples, respectively) determined by plating onto selective agars and identification by biochemical tests. For the recovery of freeze-injured *Salmonella*, there was a significant difference between TA10 broth and LAC broth (189 versus 133 positive samples, respectively). TA10 broth was more effective than not only LAC broth but also UP broth for recovery of freeze-injured *Salmonella*.

Significance: TA10 broth is more effective than LAC broth for testing of beef that may be contaminated with heat- and freeze-injured *Salmonella* spp.

P1-151 ISO 16140/MicroVal Evaluation of a Defined Medium for Enumeration of Thermotolerant *Campylobacter* spp.

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Introduction: Oxoid Brilliance™ CampyCount Agar is a novel defined medium for the direct enumeration of the thermotolerant *Campylobacter* spp., *C. jejuni* and *C. coli*. Presumptive-positive colonies grow as distinct, dark red colonies against a clear background, facilitating enumeration.

Purpose: Brilliance CampyCount Agar was evaluated in an independent MicroVal validation study according to ISO 16140:2003.

Methods: Brilliance CampyCount Agar was evaluated against modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA) for the enumeration of thermotolerant *Campylobacter* spp. from poultry samples, according to ISO 10272-2:2006. Both media were incubated at 41.5°C for 40-48 hrs in a microaerobic atmosphere. The evaluation was conducted in accordance with the quantitative methods validation section of ISO 16140:2003. In addition to the confirmation requirements of ISO 10272-2, presumptive colonies on Brilliance CampyCount Agar were confirmed using the Oxoid Dryspot *Campylobacter* Latex kit and O.B.I.S Campy test.

Results: In the method comparison study Brilliance CampyCount Agar was shown to have comparable performance to the reference method (mCCDA) in terms of inclusivity, exclusivity and limits of detection and quantification. Statistical analysis of the linearity showed no statistically significant evidence of lack of fit ($P = 0.25$). Linear regression analysis (GMFR) demonstrated the relative accuracy of the reference and alternative methods to be equivalent ($r = 0.99$, $y = 1.05 \times -0.14$) for all confirmation methods. The inter-laboratory study was conducted with 17 laboratories in 8 countries. Samples of minced chicken meat were artificially contaminated to Low ($\log_{10}$ 3.4 CFU/g), Medium ($\log_{10}$ 4.7 CFU/g) and High ($\log_{10}$ 6.0 CFU/g) levels of contamination. Results showed no significant bias between both methods at those levels (D = 0.08, 0.14, and 0.19 respectively).

Significance: Brilliance CampyCount Agar was shown to be comparable in performance to mCCDA for the enumeration of thermotolerant *Campylobacter* spp. in poultry products. Campylobacters were easier to enumerate as they were distinct, dark red colonies on a clear background. The Oxoid Dryspot *Campylobacter* Latex kit and O.B.I.S Campy tests were found to be accurate methods confirming presumptive growth on Brilliance CampyCount Agar. The certificate of compliance (2008-LR12) can be found on www.microval.org.

P1-152 Comparison of Methods for the Recovery of *Escherichia coli* O157:H7 from Leafy Greens

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Introduction: The current FDA BAM cultural method for the recovery and detection of O157:H7 and non-O157 Stx-producing *E. coli* (STEC) is based on the enrichment of produce rinses. However, the effectiveness of only rinsing produce to remove attached bacterial organisms is largely unknown.

Purpose: The objective of this study is to determine the relative effectiveness of soak and rinse methods for the recovery of *E. coli* O157:H7 from inoculated leafy greens.

Methods: Bags of baby spinach, teenage spinach, and hearts of romaine lettuce were purchased at local grocery stores, spiked with approximately 0.02 CFU/g of *E. coli* O157:H7 and subjected to the current FDA BAM method. Briefly, 20–200 g samples were each added to 625 mL of 1X BPW with pyruvate. 125 mL was removed as the rinse sample. The soak sample was the remainder and both were incubated overnight at 42°C. A portion of the rinse and soak enrichment was removed and subjected to both PCR and cultural isolation by plating serial dilutions onto TC-SMAC and R & F® *E. coli* O157:H7 agar.

Results: Three different strains of *E. coli* O157:H7 were inoculated (ATCC strain EDL-933, and two biofilm forming strains, J454 and J456) onto baby spinach, teenage spinach and hearts of romaine lettuce. In all cases, the soak procedure produced a greater number of positive samples when compared to rinsing. This was confirmed by both enrichment PCR and plating. The number of additional positive samples (for a given strain with a maximum of 20 possible) picked up with soaking ranged from 4 to 12 with a mean of 7 positive samples gained.

Significance: Concerns have been raised if an organism were present in low levels and/or were strongly attached to the produce surfaces, then it may not be possible to rinse it off into the enrichment media: thus producing false-negative results. Our data indicate a soak procedure would decrease the likelihood of false-negative results.

P1-153 Evaluation of Sample Preparation and Pre-enrichment Media on the Recovery of *Salmonella* spp. from Fresh Strawberries

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Introduction: Salmonella spp. are ubiquitous organisms. The presence of this pathogen in fruits increases the risk of illness. The sample preparation and selection of pre-enrichment broth are critical steps for the recovery of *Salmonella* from fruits. Use of non-validated procedures will produce false-negative results.

Purpose: The purpose of this study was to evaluate two methods of sample preparation and three pre-enrichment media for the recovery of *Salmonella* spp. from fresh strawberries.

Methods: Salmonella spp. isolated from farm environments (*S.* Branderup, *S.* Montevideo, *S.* Anatum, *S.* Infantis, *S.* Poona and *S.* Agona) were used to inoculate strawberries. Three inoculation levels were used: high level (10–50 MPN/25 g), low level (1–5 MPN/25 g), and uninoculated. Ten samples for each sample preparation method (rinse and soak), each pre-enrichment media (universal pre-enrichment broth, lactose broth and buffered peptone water) and each inoculation level (high, low and uninoculated) were analyzed. All samples were analyzed by the Food and Drug Administration's Bacteriological Analytical Manual culture method. Sample preparation results were compared using McNemar's test for paired data, and pre-enrichment media results were compared using chi-square test for unpaired data at significant level of 0.05.

Results: There was a significant difference between sample preparation methods ($P > 0.05$). Thirty-seven and one positive samples were obtained by soak and rinse method respectively. Thirty-six false-negative results were obtained by the rinse sample preparation method. There was not significant difference among pre-enrichment media regardless of inoculation level ($P < 0.05$). The numbers of positive samples were: 11, 12 and 14 using the universal pre-enrichment broth, lactose broth and buffered peptone water, respectively. False positive results were not obtained.

Significance: Soak method with any pre-enrichment media (buffered peptone water, lactose broth or universal pre-enrichment broth) can be used for the suitable recovery of *Salmonella* spp. from strawberries.

P1-154 Descriptive Survey of Enriched and Non-enriched Soil and Phyllosphere Samples to Improve *Salmonella* Detection Methods

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Introduction: Tomatoes have been implicated in numerous *Salmonella* illness outbreaks over the past fifteen years. At least four outbreaks of

Salmonella Newport have been traced to tomatoes grown in Accomack County of Eastern Virginia between 1998 and 2010. FDA researchers examined the microbial ecology associated with tomato crops at the Virginia Tech research station in Painter, Virginia (Accomack County) to shed light on how *Salmonella* is becoming associated with tomatoes grown in this region. Methods to recover *Salmonella* relied on pre-enrichment culturing medias that facilitate co-enrichment of numerous other species – some of which may actually be inhibiting *Salmonella*, thereby seriously reducing detection and recovery precision.

Purpose: FDA researchers characterized microflora from cultured and uncultured samples of tomato surfaces and soils from the research station in efforts to define pre- and post-enrichment microflora to better understand and minimize limitations associated with culture-based recovery methods for *Salmonella*.

Methods: DNA from soil and phyllosphere samples was extracted pre- and post-culture in Universial Pre-enrichment Broth (UPB). UPB is the first step as described in the Bacteriological Analytical Manual (BAM) for recovery of *Salmonella*. PCR products of variable regions of 16S rRNA genes were generated from both cultured and non-cultured samples of soil and phyllosphere microflora and sequenced using Roche 454 Titanium Chemistry. Taxonomic annotation was achieved using Blast with NCBI and specialized 16S rRNA gene pipelines utilizing Ribosomal Database Project taxonomy.

Results: Increased diversity of bacterial phyla was found associated with uncultured soil and phyllosphere samples. Enrichment of *Enterobacteriaceae* was observed between cultured and uncultured samples, however co-enrichment of certain clades of *Bacilli* was also evident. Of particular interest were *Bacilli* from the family Paenibacillaceae. The genus *Paenibacillus* was enriched in both phyllosphere and soil UPB cultured samples.

Significance: The co-enrichment of *Paenibacillus* by UPB is extremely significant because *Paenibacillus* has been shown to greatly inhibit Gram-negative species such as *E. coli* and *Salmonella* – often the targets of the pre-enrichments to start with. *Salmonella* was strongly inhibited by *Paenibacillus* in laboratory experiments at the FDA. This suggests that to improve the enrichment of target organisms such as *Salmonella*, selectively inhibitory additives for *Paenibacillus* should be added to UPB.

P1-155 Evaluation of Chromogenic Media for the Detection and Enumeration of *Listeria monocytogenes* and *Listeria* Species

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Introduction: Chrom ID Ottaviani Agosti (OAA, bioMérieux) is a chromogenic agar for selective isolation, enumeration and presumptive identification of *Listeria monocytogenes* and other *Listeria* species. All species of *Listeria* produce turquoise blue colonies (glucosidase activity). *L. monocytogenes* is differentiated by an opaque halo around the colony (phospholipase C activity).

Purpose: A study was conducted at Silliker Australia to validate this media for detection of *Listeria* in food and environmental samples and for enumeration of *L. monocytogenes* in food. This study was conducted as part of the AOAC Research Institute approval process.

Methods: For detection of *Listeria*, samples are enriched in Half Fraser broth for 24 h prior to plating. For enumeration, samples are diluted in Buffered Peptone Water (BPW), then inoculated directly onto spread plates. The study included validation for detection and enumeration of *Listeria* in 5 foods (cheddar cheese, lettuce, raw shrimp, crab meat and raw ground beef) and detection on 4 surfaces (stainless steel, plastic, concrete, and ceramic) by comparison to standard reference methods (USDA, AOAC, FDA BAM, as appropriate).

Results: For all foods and surfaces there was no significant difference between OAA and the reference methods for detection of *Listeria* in food and surface samples. For the enumeration study, which included 5 foods and 4 inoculation levels (0, 100, 1000 and 10,000 CFU/g) results were compared with a *t*-test at the 5% level. For most foods and levels, the methods were not significantly different and the OAA method showed better repeatability than the MPN method. Several confirmation methods were compared: API *Listeria*, Rapidec *L. mono*, Vitek 1 and Vitek 2. These bioMérieux products gave equivalent results.

Significance: OAA agar provides a simple, convenient and reliable method for detection of *Listeria* species in food and surface samples, providing a presumptive result for the presence of *Listeria* or *L. monocytogenes* within 48 h and a presumptive result for enumeration within 24 h. The method may be performed in a routine microbiology laboratory without the need for additional instrumentation. The use of proprietary confirmation methods provides further convenience and time savings.

P1-156 Differential Recovery and Survival of *Escherichia coli* O157:H7 from Inoculated Soil and Spinach Plants under Field Conditions According to Agronomic Practices

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Introduction: A better understanding of the pre-harvest fate of pathogens will depend on characterizing the influence of agronomic conditions on survival and secondary dispersal.

Purpose: To determine the influence of soil type, nitrogen dose, and spatial positioning on spinach leaves in relation to time post-contamination (DPI) by *E. coli* O157:H7 (EcO157).

Methods: Spinach was cultivated under greenhouse and field conditions June to November (2009–2010). Nitrogen regimes were 112, 224, and 336 kg/ha. Soil used in greenhouse was pasteurized for 0, 30, 60 and 90 minutes. Two *stx*-minus EcO157, Log 2.3 CFU/g soil, were inoculated into greenhouse irrigation-fertilizer solutions. Field tests used an infusion sachet at log 8 or 4 CFU/sachet. Greenhouse soil samples were 5 x 10 cm cores while field cores were 10 x 15 cm taken at 0, 10 or 20 cm from the sachet. Spinach was sprayed with log 0.3 and 0.56 CFU/m^2 of EcO157. Quantitative recovery or PCR-based detection was done separately from petioles or leaves.

Results: EcO157 persisted up to 45 days in pasteurized soils but limited to 30 days in native soil. Applying 336 kg/ha N, EcO157 was detectable in 67% of samples 60 DPI while only 40% at 112 kg/ha. Low levels of applied bacteria were detected on leaves while petioles were consistently positive. In field conditions, survival was positively correlated with soil moisture content and inoculum form. Survival limitation was associated with lower soil water content; lowest values having 50% or greater ($P < 0.05$) decrease in viable detection. Underneath the sachet, EcO157 was recovered 90 DPI. At 10 cm, 23% were positive up to 30 days. After 14 DPI, 33% of petioles were positive while only 10% for inoculated leaves.

Significance: Our findings indicate that soil water availability had a greater impact on *E. coli* O157:H7 survival than nitrogen dose. Localization of pathogen survival may provide an opportunity to redesign harvest operations towards greater risk reduction.

P1-157 Using Lateral Flow Devices for Semi-quantitative Analysis of GMOs

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Introduction: Lateral flow devices are often used for rapid, qualitative testing for the presence of GMOs in grains. These rapid tests are widely used in the field for screening purposes.

Purpose: The purpose of this study was to adapt a series of these tests for semi-quantitative use by incorporating a strip reader to analyze the results rather than relying on a visual reading, as is common practice in the qualitative methods.

Methods: These tests are lateral flow devices based upon sandwich ELISA principles. Samples are extracted with buffer, and the extracts are allowed to travel up the device. After five minutes incubation, the presence or absence of a visual marker on the device indicates the presence or absence of a selected GMO trait in the sample. The marker varies in intensity with varied concentrations of analyte in the sample. This variation in intensity of the visual marker, in conjunction with a strip reader measuring the reflectance or optical density of the line, may be used to semi-quantitatively measure the trait in a sample. Validation studies on this technology were performed for CP4 EPSPS in soy and PAT, Bt-Cry1Ab and Bt-Cry1F in corn.

Results: These methods were found to be semi-quantitative over a range of 0.1% - 4% CP4 EPSPS soy, 0.9%-4% PAT corn, and 0.5-4% Bt-Cry1Ab or Bt-Cry1F corn modified seed in unmodified seed when the test was run from 21 °C to 35 °C. Based on the results of fortified samples, GMO content can be semi-quantitatively determined within set rages (for example, 0.1-0.5%, 0.5-1.0%, 1.0-4.0%, or >4%). The limit of detection was found to be 0.1% CP4 EPSPS in soy, 0.9% PAT in corn, and 0.5% Bt-Cry1Ab and Bt-Cry1F in corn.

Significance: The use of digital-imaging software with a reader allows for objective semi-quantitative methods with results independent of the individual user's visual readings.

P1-158 Validation of On-site Rapid Methods for Food Allergen Management

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Introduction: Food allergy, an immune response to a protein present in food that the body mistakenly believes is harmful, is an important health problem in modern society. One of the major risks for food manufacturers is the potential for cross-contamination with food allergens during production processes. For this reason, allergens continue to be the largest single cause of global product recalls.

Purpose: The aim of this study was to validate AgraStrip® Allergen Test Kits, immunological rapid tests in a lateral flow format, developed for the detection of allergens in food, rinse waters and environmental swab samples.

Methods: An extracted sample is transferred to an incubation vial that contains specific ready-to-use antibodies. If the sample contains an allergen, an antigen-antibody complex will form. This is subsequently detected by means of the test strip.

Results: Extensive validation studies on a range of food matrices including yogurt, biscuits, chocolate and cooking sauces indicated low detection limits of 5 mg/kg gluten, 1 mg/kg almond protein, 1 mg/kg peanut protein and 1 mg/kg casein and showed no false positive or false negative results.

Significance: AgraStrip® Allergen Test Kits are easy to use, give a result in approximately 10 minutes and can be conducted without further equipment which is very important for on-site testing in the manufacturing facility.

P1-159 Fully Stable 13C-labeled Internal Standards for Mycotoxin Analysis

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Introduction: The popularity of LC-MS/MS methodology for analysis of mycotoxins is increasing. However, interferences from matrix components in these methods can lead to differences in analyte ionization. Ionization efficiencies can vary between matrix samples and pure standard calibrants, causing the mass spectrum to show different signal intensities. Because of this, the sample analyte peak cannot be compared to the calibration curve (made from pure standard calibrants) for concentration calculations.

Purpose: To overcome this ionization effect, 13C isotope-labeled internal standards were used. 13C-labeled mycotoxins have the same characteristics as their 12C analogues, eluting at the same retention time in chromatography. They are separated by the mass difference between the 12C and 13C mycotoxins.

Methods: The 13C peak, representing the known amount of 13C labeled mycotoxin added, can be used to calculate the unknown amount of the 12C mycotoxin. A method utilizing this technology was developed for the simultaneous detection of eight *Fusarium* mycotoxins in cereal grains, including maize and wheat. The toxins included type A and B trichothecenes and zeralenone.

Results: LODs ranged from 1 to 4 ug/L, and LOQs ranged from 2 to 20 ug/L. The %RSD of multiple repetitions of spiked samples was less than 15% overall, and most data points showed variation of less than 10%. Recoveries of the toxin from spiked matrices varied by toxin and matrix and ranged from 50 to 110%. They were also compared with a GC-ECD method. The results compared favorably, with only a 2% difference seen between the methods over concentrations ranging from 30 to 1000 ug/kg.

Significance: The use of 13C-labeled internal standards with LC-MS/MS allows for methods which are applicable to a wide variety of analytes, with no limitations by molecular mass, a straightforward sample preparation, and no chemical derivatization required. This is the basis for multi-mycotoxin analysis.

P1-160 Screening Method for Flunixin and Ceftiofur in Bovine Kidney

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Introduction: Flunixin, a non-steroidal anti-inflammatory drug, and ceftiofur, a protein bound beta-lactam antibiotic, are detected frequently in bovine tissue as reported by the FSIS. These residues are detected in inhibitory-screening-test-positive samples quarantined during slaughter. However, flunixin and ceftiofur-bound-metabolite are not inhibitory at tolerances and may elude detection. A rapid method is needed to screen for these drugs in tissue.

Purpose: To develop a lateral flow (LF) method for detecting ceftiofur and flunixin in kidney and to assess method sensitivity using spiked and incurred kidney.

Methods: Beta-lactam and flunixin test kit (LF) with Kidney Extraction Swab (KES), Negative Control (KESNC), ROSA 56C 8 min. incubator and reader were supplied by Charm Sciences, Inc. USP flunixin and penicillin G were spiked at five levels into KESNC (n = 30). One hundred micro-liters were added to KES and activated into buffer; then, 300 µl of buffer-extract was applied to LF and incubated and interpreted with reader. Frozen incurred kidney samples containing 343 ppb, 21 ppb flunixin and 1150ppb ceftiofur, supplied by FDA-CVM, were tested (n =10) along with negative kidney (n = 30) following kit instructions. Positive extracts were diluted 3 fold with KESNC extract and tested as 115 ppb and 7 ppb flunixin and 383 ppb ceftiofur.

Results: Penicillin G was detected below 50 ppb tolerance at 20–40 ppb. Flunixin was detected below the 100 ppb kidney target level at 20–80 ppb in spiking experiment. All incurred samples (10/10) tested positive for ceftiofur at 1150 ppb and 383 ppb levels (400 ppb tolerance). All (10/10) samples containing flunixin at 21 ppb, 115 ppb and 343 ppb levels tested positive, while (7/10) containing 7 ppb were positive (25 ppb tolerance). No false positives (0/30) were found for the negative kidneys.

Significance: The easy 10-minute procedure detects ceftiofur and flunixin below or near tissue tolerances. This addresses a residue detection gap in slaughterhouse-inhibition-tests providing additional consumer protection.

P1-161 Development of an Optimized Method for the Recovery of Viable F-RNA Coliphage MS2 from Meat

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Introduction: F-RNA coliphages, part of the gut flora and likely to be deposited on meat along with other enteric organisms during carcass dressing and processing, may be regarded as an indicator and/or surrogate for potential zoonotic enteric viruses. There is no recommended sampling method for viruses on meats and there is a lack of information on the attachment of enteric viruses or F-RNA coliphages to gauze swabs, cellulose sponges and muscle and fat tissue.

Purpose: The objective was to optimize the recovery of MS2 from muscle and fat tissue of meat by comparing different eluents, homogenization methods and sampling techniques to obtain an optimal method for their recovery from meats.

Methods: MS2, inoculated at levels of 1 x 10^4 plaque forming units, was enumerated using a plaque assay. The effect of 3 different eluents (phosphate buffered saline (PBS), 10% beef extract pH 7.2 (BE), and tryptose phosphate broth (2.9%) with 6% glycine pH 9.5 (TPB)) and 3 different homogenization methods (stomacher, stomacher combined with sonication, and pulsifier) were compared. The sampling techniques of excision, swabbing with gauze or cellulose sponges were compared with homogenizing the entire inoculated muscle or fat surface.

Results: The recovery of MS2 from cellulose sponges using BE was significantly higher ($P < 0.05$) than TPB which was significantly higher than PBS. No significant differences were observed between the different eluents with gauze and muscle or between the homogenization methods. When MS2 was recovered from muscle tissue with BE, the sampling techniques of homogenizing the entire sample (56%) was equal to excision (43%) and swabbing with a cellulose sponge (38%) which were significantly higher than swabbing with gauze (28%). A second grouping of means indicated that homogenizing the entire sample was significantly higher than the other 3 sampling techniques. When MS2 was recovered from fat, homogenizing the entire sample (78%) was equal to excision (74%), which were significantly higher than swabbing with gauze (49%) or cellulose sponge (29%).

Significance: The recovery of F-RNA coliphages from meat is affected by the sampling technique. When choosing a nondestructive sampling method such as a cellulose sponge, a higher recovery can be obtained with BE as an eluent.

P1-162 Evaluation of Pre-enrichment Procedures to Recover Select *Salmonella* Serovars from Artificially Contaminated Test Portions of Curly Parsley, Cilantro and Basil with the U.S. Food and Drug Administration BAM *Salmonella* Culture Method

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Introduction: Salmonella spp. are commonly identified bacterial pathogens in foodborne disease outbreaks and recently, have become increasingly associated with fresh leafy green vegetables. Contamination can occur through a variety of routes and at any point in the farm-to-fork continuum; however, most likely scenarios involve contact by contaminated fecal material from any of several potential animal reservoirs. The increase in the popularity of consuming vegetables in a raw or unprepared state likely contributes to the incidence of *Salmonella* foodborne infections linked to leafy green vegetables as heating is usually sufficient to kill existing bacterial pathogens on contaminated vegetables.

Purpose: To evaluate pre-enrichment procedures to recover *Salmonella* from curly parsley, cilantro and basil with the Bacteriological Analytical Manual *Salmonella* Culture Method and to identify a pre-enrichment procedure most effective for the recovery of *Salmonella* from these leafy green produce types

Methods: Artificially contaminated test portions were evaluated with 3 pre-enrichment procedures including soak, blend and stomaching. The soak procedure included addition of the test portions to the lactose broth; the blend procedure was performed by blending test portions with the lactose broth, and stomaching, by stomaching test portions with the lactose broth.

Results: No statistically significant differences occurred between the procedures for the recovery of *S.* Typhimurium and *S.* Michigan from curly parsley ($P > 0.05$). The soak and stomach procedures were significantly more effective than blending in 1 of 2 experimental trials performed with *S.* Aba and culantro ($P < 0.05$), and the stomach procedure was more effective for the recovery of *S.* Agona from basil in 1 of 2 experimental trials. In the remaining trials, no statistically significant differences occurred between the procedures ($P > 0.05$).

Significance: Soaking or stomaching of test portions offers the most effective pre-enrichment procedure to recover *Salmonella* from curly parsley, cilantro and basil with the BAM *Salmonella* culture method.

P1-163 Development of a New Device for Rapid Detection of Total Aerobic Mesophilic Bacteria

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Introduction: A device was developed to detect fermenting and non-fermenting bacterial contamination of products with the majority of bacteria detected in < 24 hr. The device consists of a new Soleris vial system containing a mixture of carbon dioxide indicators homogeneously dispersed in a semi-permeable matrix separated by a gas permeable membrane from a chamber containing growth media and sample. The carbon dioxide indicators are located in a region of the device with an optically transparent window that permits spectroscopic detection of the absorbance change that occurs upon detection of carbon dioxide produced from bacterial metabolism.

Purpose: The purpose of this study was to develop a rapid detection device for total aerobic mesophilic microorganisms and test the ability of the device to detect bacterial growth in a variety of food matrices.

Methods: Initial experiments were conducted to optimize the indicator and gas permeable membrane chemistries of the device to rapidly detect carbon dioxide and prevent changes in sample or media pH from altering the indicator. Detection speeds in these experiments were evaluated by exposing devices to several concentrations of carbon dioxide and spectroscopically measuring the change in optical density. For inclusivity studies, the ability of the device to detect a panel of non-fermenting bacterial strains was evaluated by inoculating growth media at < 100 CFU/mL and spectroscopically measuring detection times at 35°C. In addition, the relationship between detection speed and initial bacterial load was evaluated by preparing a series of vials containing media and UHT milk at several inoculums and measuring detection speeds.

Results: A device was constructed that rapidly detected carbon dioxide and was not affected by changes in media pH due to sample. Inclusivity testing indicated all the bacterial strains of interest were detected in < 24 hr. In addition, detection speed was linearly correlated to bacterial load in UHT milk samples inoculated from 10^9 to 10^2 CFU/mL. The device was unaffected by changes in media pH due to sample.

Significance: The device provides a system to detect fermenting and non-fermenting bacterial contamination in < 24 hr for a wide variety of food products.

P1-164 Development of Single-walled Carbon Nanotube (SWNT) Sensors in Application to Thermal Oxidation of Frying Oils

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Introduction: Modern people are exposed to many kinds of fast food and street food. So maintaining the proper state of frying oils used for them is a very important factor in food safety. However, an adequate monitoring for frying oils' rancidity hasn't been done well and chemical measurement methods currently used take lots of time and require expertise.

Purpose: SWNT sensors are chemiresistors which are fabricated and coated with polyethylenimine (PEI) to enhance sensitivity and selectivity of the sensors. The resistance of the thermally oxidized oils was measured based on acid value of the oils. PEI functionalized SWNTs are known to have high selectivity for strong oxidizing agents. Development of a device using SWNT sensors are expected to measure in real-time, on site with a wide range and high selectivity.

Methods: The equipment consists of three SWNT sensors and one temperature sensor. The measurement of was conducted for 5min in 100°C and recovery time was conducted for 10 min in room temperature. Frying oils were taken in 180°C up to acid value 3.7. The chemical methods are selected to determine acid value and TPM (total polar materials). Frying oils were taken in 160°C, 180°C and 200°C for 336 hours (24 h interval). All experiments were carried out three times and the values are handled statistically.

Results: We confirmed co-relationship between values from chemical methods and SWNT sensors. As the acid value of oils was increased, the resistance of sensors was decreased. There is no significant difference between acid values by chemical method and SWNT sensor values.

Significance: By determining oil's oxidation level in real time and on site, it's possible to change frying oils at proper timing. It's expected to contribute to public health by reducing consumption of oxidized oil.

P2-01 Application of an Optimized, Automated Nucleic Acid Extraction Procedure for qPCR Detection of *Escherichia coli* O157:H7 in Artificially Contaminated Napa Cabbage, Green Onions and Zucchini

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Introduction: E. coli O157:H7 is an important foodborne pathogen, capable of causing serious illness in humans. Use of rapid molecular assays such as qPCR can offer improved screening over other methods. However, efficient extraction of nucleic acid is essential to effectively using downstream qPCR assays.

Purpose: The purpose of this study was to optimize and apply a nucleic acid extraction procedure from enrichments of produce samples inoculated with *E. coli* O157:H7 for use with two different qPCR assays.

Methods: Zucchini (~125 g), green onion (200 g) and napa cabbage (200 g) were artificially inoculated (n = 6) with low (~0.1 CFU/g), high (~1.0 CFU/g) and no *E. coli* O157:H7 and enriched in mBPWp broth (1.5 x zucchini wt; 125 ml rinsate +125 ml 2 x mBPWp for green onion and cabbage) overnight as per FDA Bacteriological Analytical Manual. Enrichments were screened by two multiplex qPCR methods (*stx1, stx2, IC* and either O157:H7 *uidA* SNP or *wzy* O157 gene targets). Prior to qPCR, DNA was extracted either using standard wash, spin, boil (WSB) method or optimized MagNA Pure Compact (MPC) procedure.

Results: qPCR detection of *E. coli* O157:H7 was successful in all foods at both high and low inoculation levels using all combinations of DNA extraction procedures and multiplex assays. All controls performed satisfactorily as well. Although this is a qualitative method, a closer look at cycle threshold (Ct) values shows significantly greater amounts of DNA (lower Ct) extracted using the MPC for all foods and in all gene targets (ΔAvg Ct shift = 4.93, 6.29, 5.04 *stx2, uidA, stx1*, respectively; 7.15, 7.07, 6.42 *stx2, wzy, stx1*, respectively) than for WSB extraction. Comparing both qPCR assays, the *wzy* target showed slightly lower Ct values than the *uidA* target (ΔAvg Ct = 2.27 and 3.05 for WSB and MPC, respectively).

Significance: Rapid qPCR detection of *E. coli* O157:H7 offers a quick, sensitive screening procedure to increase food safety. Use of semiautomated nucleic acid extraction procedures (MPC) can potentially offer greater sensitivity in qPCR assays than the basic wash and boil technique.

P2-02 Validation of a Hot-start Modification to a Commercial PCR Assay for Detecting *Salmonella* in Foods

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Introduction: Salmonella is found in many food and environmental sources, and it can cause serious illness. In the presence of competing flora, culture isolation may require lengthy procedures with skilled interpretation. Rapid non-culture methods, such as those based on PCR, can provide for fast and accurate detection. Under abuse conditions, however, PCR can generate non-specific amplified product that may lead to atypical results requiring culture confirmation with potential delays in shipping of product. A hot-start functionality that becomes inactive after cycling begins can eliminate non-specific priming at temperatures prior to the start of PCR reducing or eliminating atypical results.

Purpose: This study evaluated the inclusivity, exclusivity, and effectiveness of a commercial PCR assay for detecting *Salmonella* that was modified to include hot-start functionality.

Methods: Inclusivity testing was performed at ~10^5 CFU/mL, while exclusivity testing was performed at ~10^8 CFU/mL. Artificially contaminated dry pet food, ground beef, cream cheese, frankfurters, and orange juice were tested and results compared with the appropriate USDA or FDA reference method. Samples were inoculated with *Salmonella* at levels expected to yield fractional results based on preparatory studies. Samples were enriched in the appropriate reference method's primary enrichment media (LB, BPW, or UPB) and corresponding samples were enriched in the test method's proprietary media. Secondary enrichment and culture confirmation were conducted using the appropriate reference method.

Results: For inclusivity/exclusivity testing, 364 target strains tested for the original version of the kit were all found to be reactive using the modified test kit, while 46 non-target strains were negative. For effectiveness testing, which included 200 spiked and 50 unspiked samples for test and reference methods, 37/100 spiked samples from the reference method enrichments were PCR-positive, while 44/100 spiked samples from the proprietary enrichment were PCR-positive. All negative controls were negative by PCR. All PCR-positive samples culture confirmed, and all PCR-negative samples were negative by culture.

Significance: This study indicated that PCR detection of *Salmonella* using the test kit modified with hot-start technology is rapid and sensitive. Test kit results demonstrated no significant difference when compared with the reference culture methods.

P2-03 The Importance of Mechanical Lysis and Inhibitor Removal in DNA Extraction and Detection of a Foodborne Pathogen *(Listeria monocytogenes)*

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Introduction: To maintain a safe and pathogen-free food supply, there is an increasing demand for timely and economical evaluation of products within the food industry. This has led to DNA extraction methods that are fast but crude. DNA extraction after a sample pre-enrichment step is typical. However, the presence of competing microorganisms can result in a mixed population that impacts the ability to detect the organism of interest especially if they are present in low numbers. For this reason sensitive detection can be dependent on efficient DNA extraction and purification of a sample.

Purpose: The standard method used for DNA extraction from food cultures is the FDA approved Wash+Boil protocol which results in a crude preparation. The goal of this project was to compare lysis and inhibitor removal ability of a commercial extraction method with the FDA approved Wash+Boil method for *Listeria monocytogenes* spiked into mascarpone cheese and dark chocolate.

Methods: A commercial lysis method was modified to compare chemical lysis with either bead beating or heating. Purification methodologies were also modified to compare silica spin columns to magnetic beads. The effects of inhibition carry over on DNA amplification was assessed by comparing detection of DNA purified both with and without Inhibitor Removal Technology (IRT). DNA yields, purity, and detection sensitivity from all modifications were evaluated alongside the Wash+Boil method.

Results: The importance of mechanical lysis in place of heating or sample boiling was demonstrated by quantitative PCR (qPCR). DNA sensitivity was improved by over 9 Cqs or 1000 fold when bead beating was used regardless of purification method. Inhibitors were present only in DNA extracted from chocolate cultures but not cheese cultures and only in DNA purified using magnetic beads. DNA purified from contaminated chocolate using magnetic beads had a Cq=13 using IRT compared to no amplification of samples purified in the absence of IRT.

Significance: The detection of bacterial contamination of foods can be severely impacted when extraction methods are not optimal for the lysis of the organism or for removal of PCR inhibitors. A more robust standard method that is optimized for both gram positive and gram negative bacteria and inhibitory substances may be required for appropriate diagnosis of food contamination.

P2-04 Simultaneous Identification of 60 *Listeria* spp. Isolated from Meat Products Using Multiplex PCR and VITEK 2 Compact System

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Introduction: The genus *Listeria* is divided into six different species including *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri*. Especially, *L. monocytogenes* is a foodborne pathogen causing listeriosis in human beings and livestock. However, it is difficult to identify the six *Listeria* spp. only by conventional detection methods using selective media and biochemical tests. It is required to develop new detection methods for accurate and rapid identification of *Listeria* spp. in processed foods to species level.

Purpose: The objective of this study was to develop a detection method for simultaneous identification of six *Listeria* spp. in bacterial isolates of meat products using VITEK 2 compact system and multiplex PCR.

Methods: Bacterial strains were isolated from meat products in the markets using *Listeria* selection media. The presumptive *Listeria* spp. from the products were selected and identified using VITEK 2 compact system. Subsequently, a multiplex PCR using one *Listeria*-specific primer pair and six specific primer pairs for individual six *Listeria* spp. was performed to confirm the results of the VITEK system.

Results: The multiplex PCR yielded seven PCR amplicons with different size: 370 bp for all six *Listeria* species-specific; 272 bp for L. grayi-specific; 836 bp for *L. innocua*-specific; 463 bp for *L. ivanovii*-specific; 132 bp for *L. monocytogenes*-specific; 184 bp for *L. seeligeri*-specific; and 608 bp for *L. welshimeri*-specific. Sixty-one bacterial isolates were identified as *Listeria* spp. using VITEK 2 compact system. However, 60 isolates except one strain were identified as *Listeria* spp. using the multiplex PCR. Moreover, 41 out of 60 *Listeria* spp. identified using the multiplex PCR were matched to the results of the VITEK system.

Significance: These results suggest that the multiplex PCR developed in this study can be successfully employed to identify six *Listeria* spp. in the isolates from processed foods to species level.

P2-05 Application of the AdvanCE FS96 Parallel Capillary Electrophoresis Instrument and DNA PROsize™ Program for DNA-based Typing of *Salmonella*

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Introduction: A variety of DNA fragment-based methods have been developed for pathogen characterization, fingerprinting and tracking. These include Random Amplification of Polymorphic DNA (RAPD), Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR), Repetitive Extragenic Palindromic sequence PCR (rep-PCR) and others. Reliable, reproducible and sensitive means for separating, detecting and analyzing the resultant fragment pools are needed in order to fully realize the power of these typing or fingerprinting approaches.

Purpose: To evaluate the use of a commercial parallel capillary electrophoresis (PCE) instrument for detection, resolution and analysis of DNA fragments obtained via ERIC-, RAPD- and rep-PCR of *Salmonella* spp.

Methods: DNA fragments from a panel of *Salmonella* spp. were generated using published ERIC-, rep- and RAPD-PCR protocols, then loaded for analysis directly onto an AdvanCE FS96 PCE instrument (Advanced Analytical, Ames, IA). Because FS96 gels contain an intercalating DNA dye, no independent staining step was required. Fragments were separated at 9 kV for 90 min, with 10- and 20,000-base DNA size markers used to define upper and lower size limits to enable data normalization. Parallel experiments using traditional slab gels were also conducted. All experiments were performed in triplicate.

Results: PCE analysis of ERIC-, rep- and RAPD-PCR resulted in clear peaks, with a range of 30-50 peaks resolved. Faint peaks not visible on traditional slab gels were detected using PCE. Pattern recognition software (DNA PROsize™, Advanced Analytical) was used to directly analyze results and facilitated comparison of fragment patterns across *Salmonella* isolates and determination of reproducibility across experiments.

Significance: Traditional slab gel analyses of DNA fragments can vary greatly across experiments and require lengthy manual interventions such as gel imaging and digitization. Use of PCE and subsequent analysis with pattern recognition software enabled reproducible, high-resolution separation and analysis of DNA fragments without the need for gel staining or lengthy image analysis procedures.

P2-06 Development of a Foodborne Bacterial Pathogen PCR Electrospray Ionization Mass Spectrometry Biosensor Detection Assay for Rapid, High-throughput Food Testing

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Introduction: Methods that reduce the time of detection, identification and discrimination of pathogenic foodborne bacterial pathogens are in great demand by industry and regulators both domestically and internationally. Molecular methods that focus on multiple DNA targets may allow for highly specific identification of bacterial species.

Purpose: To this end, we are reporting on the development and initial evaluation of a PCR/ESI-MS (electrospray ionization mass spectrometry) based biosensor assay for rapid detection of 4 important foodborne pathogens.

Methods: This assay uses 8 primer pairs to detect and differentiate *Salmonella* spp. *Escherichia coli*, *Shigella* spp. and *Listeria* spp. These primers bind to conservative regions in *mutS*, *mdh* in *Enterobacteriaceae* and *prfA* and *iap* in *Listeria*.

Results: Initial work built a DNA basecount database that contains over 140 *Salmonella*, 139 *E. coli*, 11 *Shigella*, 36 *Listeria* patterns and 18 other *Enterobacteriaceae* organisms. Evaluations using FDA SAFE *Salmonella* panel confirmed the assay's ability to sub-speciate *Salmonella*. Surprisingly, some differentiation of *S. enterica* subsp. *enterica* into individual serovars or clusters containing 2 to 6 serovars was also observed. Tomato spiking experiments investigated the limit of detection in a food matrix. At 24 hr incubation, the assay was able to detect *Salmonella* at a pre- pre-enrichment (PPE) spike of 1 CFU/ml, similar to a conventional multiplex PCR. When the incubation period was reduced to 6 hr the PCR/ESI-MS assay could detect *Salmonella* at 20 CFU/ml PPE spike, whereas conventional PCR did not detect *Samonella* until 2 x10^4 CFU/ml PPE spike.

Significance: This work demonstrates that the foodborne bacterial pathogen PCR/ESI-MS assay is capable of detecting and subtyping *Salmonella*, *E. coli*, *Shigella* and *Listeria*. Also the assay can detect the presence of *Salmonella* in a food matrix, specifically tomatoes. Current work is focusing on detection of *Salmonella* in other food matrices and the use of this assay in environmental surveillance samples.

P2-07 Development and Evaluation of Novel One-step TaqMan Realtime RT-PCR Assays for the Detection and Direct Genotyping of Genogroup I and II Noroviruses

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Introduction: Current detection and genotyping methods of genogroup (G) I and II noroviruses (NoVs) consist of a 2-step approach including detection of viral RNA by TaqMan realtime RT-PCR (RT-qPCR) followed by conventional RT-PCR and sequencing of partial regions of ORF1 or ORF2.

Purpose: To develop novel long-template one-step TaqMan assays (L-RT-qPCR) for the rapid detection and direct genotyping of GI and GII NoVs and to evaluate the sensitivity and specificity of the assays.

Methods: GI and GII-specific broadly reactive L-RT-qPCR assays were developed by combining existing NoV primers and probes targeting the open reading frame (ORF)1-ORF2 junction as well as region C at the 5′-ORF2. The assays were validated using GI and GII RNA transcripts and a coded panel of 75 stool samples containing NoV strains representing 9 GI genotypes and 12 GII genotypes, as well as sapoviruses, astroviruses, polioviruses, and rotaviruses. L-RT-qPCR products were typed by sequencing.

Results: The novel GI and GII L-RT-qPCR assays detected and typed all but one of the NoV positive panel samples. As few as 5-500 RNA copies could be accurately typed by sequencing of amplicons.

Significance: The one-step TaqMan RT-qPCR assays were shown to be sensitive for detection and direct genotyping of GI and GII NoVs from clinical and environmental matrices.

P2-08 *Salmonella* Detection in Meat: Comparative and Collaborative Validation of a Non-complex and Cost Effective Pre-PCR Protocol

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Introduction: Cost-effective and rapid monitoring of *Salmonella* in the meat production chain can contribute to food safety.

Purpose: The objective was, for the first time, to validate an easy-to-use pre-PCR sample preparation method based on a simple boiling protocol for screening of *Salmonella* in meat and carcass swab samples using a real-time PCR method.

Methods: The protocol included incubation in buffered peptone water, centrifugation of an aliquot and a boiling procedure. The validation study included comparative and collaborative trials recommended by the Nordic Organization for Validation of Alternative Methods (NordVal). The comparative trial was performed against a culture based reference method (NMKL187, 2007) and a previously NordVal-approved PCR method with a semi-automated magnetic bead-based DNA extraction step using 122 artificially contaminated samples.

Results: The limit of detection (LOD50) was found to be 3.0, 3.2 and 3.4 CFU/sample for the boiling, magnetic bead-based and NMKL187 methods, respectively. When comparing the boiling method with the magnetic beads, the relative accuracy (AC), relative sensitivity (SE) and relative specificity (SP) were found to be 98%, 102% and 98%, respectively (Cohen's kappa index 0.95). When comparing results obtained by the boiling to the culture based method, the AC, SE and SP were found to be 98%, 102% and 98%, respectively (kappa index 0.93). In the collaborative trial, valid results from 11 laboratories were included and apart from two false positive samples, no deviating results were obtained (SP, SE and AC were 100%, 95% and 97%, respectively).

Significance: This test is under implementation by the Danish meat industry, and can be useful for screening of large number of samples in the meat production, especially for fast release of minced meat with short shelf life.

P2-09 Propidium monoazide-qPCR Quantitation of *Escherichia coli* O157:H7 Receiving 10D Thermal Processes at 60, 70, 80, 90 and 100°C

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Introduction: PCR-based detection and quantitation methods for foodborne pathogens are limited by their inability to discriminate between viable and non-viable cells. The use of a selectively permeable DNA intercalating agent such as propidium monoazide has been proposed as a solution. It is believed that the permeability of PMA is based upon irreversible physical damage to the cell membrane. Harsh processes result in greater damage to the cell membrane and increase the efficiency of permeation of DNA intercalating agents. Previous reports have utilized harsh (boiling for several minutes) thermal inactivation of target pathogens thus ensuring maximum PMA permeation.

Purpose: To determine the accuracy of PMA-qPCR quantitation *E. coli* O157:H7 (Z = 4.4°C) following a 10D process at various temperatures.

Methods: Cells ($\log_{10}$ 9.1 CFU/mL) were thermally inactivated prior to treatment with 40 µM PMA. Genomic DNA was isolated and qPCR (SYBR Green) was performed using primers targeting the *stx1* gene and yielding an amplicon of 275 base pairs.

Results: Complete inactivation of the starting population was verified by failure to produce visible turbidity in TSB-YE at 35°C after 72 h. Amplification of serially diluted, purified DNA resulted in a theoretical detection limit of 100 CFU/mL. Although no remaining viable cells were evident by conventional culture at 60 and 70°C thermal processes, PMA-qPCR estimated the viable population at $\log_{10}$ 8.0 ± 0.2 and 8.1 ± 0.2 CFU/mL respectively. At 80, 90 and 100°C noticeable shifts in the C_T values were observed with resulting viable population estimates of $\log_{10}$ 6.6 ± 0.2, 4.8 ± 0.2, and 5.0 ± 0.6 CFU/mL respectively. Prolonged heating (90°C for 30 min) resulted in a shift in C_T value of 16.7 ± 2.3 which equates to a 4.1 log decrease in the viable population.

Significance: PMA-qPCR consistently overestimated the viable population of *E. coli* O157:H7 following mild thermal inactivation and should be limited to products that undergo prolonged exposure to high temperatures which is necessary to achieve the level of membrane damage required for sufficient permeation of PMA and accurate assessment of the remaining viable population. Improvements in PMA methods and protocols will be required before it can be used reliably to detect/quantitate viable target organisms when present with non-viable cells in food matrices.

P2-10 SMM-system: A Mining Tool to Identify Specific Markers in *Salmonella enterica*

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Introduction: Salmonella is one of the most important foodborne pathogens. Gene targets such as *invA, fimA* and other genes in *Salmonella*, have contributed to the control of this pathogen. However, some of these genes have been reported to be absent in a few *Salmonella* serovars, whereas others led to false positive results when detection methods based upon them were used for testing against non-*Salmonella* strains. To reduce the potential of false positive and/or negative misidentification caused by target sequence variation, it is necessary to mine and evaluate new specific targets of *S. enterica*.

Purpose: The aim of this study was to develop a high-throughput specific markers generation tool (SMM-system), and to mine *S. enterica*-specific coding sequences (CDSs) using this tool.

Methods: SMM-system was a visualized tool written with Microsoft Visual Studio 2008, and integrated with BLASTN program. Using SMM-system, all 4409 CDSs of *S. enterica* Choleraesuis SC-B67 were searched against a database of 16 *S. enterica* genomic sequences except for *S. enterica* Choleraesuis SC-B67. The CDSs that matched all 16 *S. enterica* genomes were selected for further comparison with 796 non-*S. enterica* bacterial genome sequences. The CDS with the lowest e-value more than 0.001 was finally identified as a *S. enterica*-specific marker. Primers were designed for a part of *S. enterica*-specific CDSs. All 30 non-*S. enterica* strains were used for pre-screening of the specificity of primer pairs. If a primer pair failed to generate an amplicon from all 30 non-*S. enterica* tested, it was then tested against a total of 101 strains of *S. enterica*.

Results: The design of the interface of SMM-system included four functional menus such as "Database Handle", "Blastn Alignment", "Genome Cutting" and "Screening". SMM-system can help users to implement various personalized pre- and post-BLASTN tasks for mining specific markers. A total of 214 CDSs were identified as specific to *S. enterica*. Eighteen primer sets were designed based on eighteen *S. enterica*-specific CDSs, respectively, and used for pre-screening of the specificity. Only seven primer sets exhibited 100% inclusivity for 101 *Salmonella* genomes and 100% exclusivity of 30 non-*S. enterica* genomes, and all seven PCR assays yielded positive results in artificially-contaminated milks.

Significance: SMM-system was a high-throughput, specific markers generation tool which can generate genus-, species-, serogroup- and even serovar-specific DNA sequences. SMM-system can be downloaded freely from http://foodsafety.sjtu.edu.cn/SMM-system.html.

P2-11 Rapid Quantitative Analysis of *Listeria monocytogenes* by Real-time PCR of Meat Processed Products in Korea

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Introduction: Listeria monocytogenes is a foodborne pathogen of concern for the food industry, which has the potential of causing human listeriosis. It is a ubiquitous microorganism, commonly isolated from foods of animal origin (e.g., raw milk, meat, poultry, fish and livestock products) such as ham, sausage, ground meat and milk product, as well as from foods of vegetable origin like salad.

Purpose: The purpose of this study was to construct the standard curves for the quantitative detection of *L. monocytogenes* by real-time PCR in processed meat products.

Methods: Ten grams of ham, sausage, ground meat and dry-sliced meat samples and 49 ml of fraser broth were added in six filtered stomacher bags. For artificial infection, *L. monocytogenes* ATCC 19115 was cultured at 37°C for 24 hr, then 10-fold serially diluted, with a final concentration of 10^0~10^5 CFU/ml. Each dilution was inoculated in stomacher bags and homogenized in the stomacher for 60 sec. All homogenized samples were transferred to a glass bottle and incubated at 37°C for 3 hr. Then 1ml of each homogenized sample and serially diluted *L. monocytogenes* was added to an Eppendorf tube, respectively, and used to extract DNA by PrepSeq rapid spin sample preparation kit (ABI, USA). Real-time PCR for construction of *L. monocytogenes* standard curves was performed with designed oligonucleotide primers and probe pair for the *L. monocytogenes inlA* gene.

Results: When *L. monocytogenes* were artificially inoculated in 10 g samples, the cell concentration of range was approximately 3.5 x 10^4 to 3.0 x 10^0 CFU/g. The standard curve of serially diluted cells was log linear for five orders of magnitude from 10^1 to 10^5 CFU/ml of *L. monocytogenes*. When four types of food matrices were artificially contaminated with serially diluted cells of *L. monocytogenes*, the detection range was from 10^4 to 3 CFU/g for ham and sausage samples, and from 10^4 to 10 CFU/g for ground processed meat and dry-sliced meat samples, respectively. The efficiency of the reaction for ham, sausage, ground meat and dry-sliced meat samples was 160%, 93%, 105% and 130%, respectively. Correlation and coefficient in real-time PCR were over 0.97 in all types of samples.

Significance: These studies suggest that real-time PCR could be useful as a complementary technique for the rapid quantification of *L. monocytogenes* down to 3 CFU/g in ham and sausage samples and 10 CFU/g in ground meat and dry-sliced meat samples.

P2-12 Detection of *Escherichia coli* O145 in Sheep Feces by Use of Two Real-time PCRs and an Immunomagnetic Separation-ELISA Method

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Introduction: Non-O157 VTEC may be difficult to detect and there are very few standardized methods for the detection of these organisms.

Purpose: In a study of verocytotoxin-producing *Escherichia coli* in Norwegian sheep, three methods were used for the detection of *E. coli* O145.

Methods: Five composite samples, consisting of feces from 10 animals, were tested from 9 sheep flocks giving a total of 45 samples. The fecal samples were enriched in buffered peptone water at 41.5°C for 16–18 h prior to AIMS-ELISA (automated immunomagnetic separation-ELISA). DNA extraction was performed simultaneously on aliquots of the enriched samples and real-time PCR carried out using two different primer and probe sets described by Perelle et al. and Fratamico et al., respectively.

Results: Using an initial cut-off for the AIMS-ELISA at an absorbance value (ABS405 nm) of 0.3 resulted in 19 positive samples. However, eight samples returning values between 0.2 and 0.3 were also plated out to accommodate low target levels. *E. coli* O145 was isolated and confirmed from seven of the samples with ABS>0.3 and one sample with ABS=0.247. For the eight culture-confirmed samples, the PCR results were variable. One of the AIMS-ELISA culture-confirmed samples was negative by both PCRs, while others again were clearly positive by AIMS-ELISA and the PCRs. In general, it was observed that the Ct-values were quite high, even for some of the positive samples.

Significance: The results indicate that none of the methods are optimal for the detection of *E. coli* O145 in fecal samples due to a rather high fraction of false-positive results that will increase the work load and costs of the methods. However, from the results presented here, the AIMS-ELISA assay was the most suitable for these samples.

P2-13 Biofilm Formation Potential of *Escherichia coli* Isolated from Water and Lettuce

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Introduction: The surface of plants is a harsh environment for fecal bacteria, with little nutrients available and exposure to environmental stress. It is known that bacteria in biofilms are more tolerant to stress than planktonic cells. In a previous project on water as a source of foodborne pathogens on lettuce, a total of 355 isolates of generic *Escherichia coli* were obtained from irrigation water (292), lettuce (42) and samples from washing and packaging facilities (21 isolates).

Purpose: The objective of the work was to study the biofilm formation potential of the isolates in vitro.

Methods: The isolates were tested in LB wo/NaCl at 37°C, 15°C and 8°C for three, five and seven days, respectively, by the use of the peg assay in microtiter plates. Triplets of each sample were used. After incubation, the pegs were dyed with crystal violet, washed and the dye was removed from the pegs prior to reading of absorbance values at 595 nm (ABS595). The temperatures were chosen to represent the intestinal environment (37°C), and also the daily mean temperature in a warm and cold Norwegian summer (15°C and 8°C).

Results: The results indicated that the biofilm production ability was highest at 15°C for 5 days, where 18%, 9.5% and 14% of the strains isolated from water, lettuce and the environment were considered as good biofilm-producing isolates (ABS595>1.0). Biofilm production was poorest at 8°C for seven days where 99% of the isolates were considered poor biofilm producers (ABS595<0.5). However, the variability was large, within the triplets and within the groups of isolates. Only a few isolates were considered as good and medium biofilm producers at more than one temperature.

Significance: The results indicate that *E. coli* isolated from water, lettuce and packaging environment are able to produce biofilm in vitro, thus creating a protective environment where fecal pathogens may survive and proliferate.

P2-14 Comparison of Culture Methods and Real-time PCR for Detection of *Salmonella* on Egg Shells and in Egg Content

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Introduction: Salmonellosis is one of the most infectious diseases in the world and is a common cause of gastrointestinal food poisoning. The recent *Salmonella* outbreaks associated with eggs have increased great attention in Korea as well as in the U.S. However, there are few studies on the application and evaluation of rapid detection method such as real-time PCR for detection foodborne pathogen in shell eggs.

Purpose: The objectives of this study were to compare sensitivity of culture methods and real-time PCR to detect *Salmonella* in commercial graded and non-graded shell eggs in Korea.

Methods: Real-time PCR assay was evaluated for the detection of major foodborne pathogens, *Salmonella* Enteritidis (SE), in shell eggs and compared to the standard culture method. The bulk 50 samples containing the contents of 20 eggs (1200 g each) were thoroughly mixed manually. All samples were analyzed by both real-time PCR and culture method.

Results: As a result of aerobic plate count, the level of background flora in graded shell eggs was lower than in non-graded shell eggs. In egg content samples, the culture method and real-time PCR detected *Salmonella* equally well, and statistical significant difference was not observed between the two methods. In graded eggs, real-time PCR similarly identified 1 out of 50 samples (2%) as positive, and it yielded a 100% correlation with results obtained by a conventional culture method. However, the culturing method also identified *Escherichia coli*, *E. fergusonii*, and *Ent. sakazaki*. from the non-graded egg shells. In egg contents, an isolate of *Salmonella* Gallinarum was found in internal contents from graded eggs and *E. coli* and *Ent. cloacae* were isolated from the non-graded eggs. From the shell surfaces, *Salmonella* spp. were not detected from any of the graded eggs. *Escherichia coli*, *E. fergusonii*, and *Ent. sakazaki* were identified from the non-graded egg shells.

Significance: Real-time PCR assay was less affected by background flora and more sensitive and specific in detecting low numbers of *Salmonella* than the culture method. For further improvement of culture method and real-time PCR, additional measures will be need to increase the number of *Salmonella* to detectable levels in the sample.

P2-15 Evaluation of a New, Single-step *Listeria* Enrichment Broth by Quantitative Polymerase Chain Reaction with Complex Food Samples

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Introduction: Testing for *Listeria monocytogenes* is an important component of food safety programs, but current methods are perceived to be complicated, lengthy or expensive. To address the need for a less complicated, more rugged, and faster enrichment process, a new selective growth medium was designed to utilize a single-step enrichment method in less time.

Purpose: Evaluate a new, single-step *Listeria* enrichment broth for the recovery of *L. monocytogenes* from fractionally spiked complex food samples using Quantitative Polymerase Chain Reaction (qPCR).

Methods: Fractional recovery studies were performed by testing 20–25 g portions of two different enriched sample matrices (deli-style turkey and potato salad) that were artificially contaminated at a low level and then held refrigerated for several days. Deli-style turkey was spiked with *Listeria monocytogenes* ATCC 7644 (5.25 CFU/25 g, determined by MPN) and potato salad was spiked with *L. monocytogenes* ATCC 19151 (0.9 CFU/25 g, determined by MPN). In addition, five non-inoculated portions were tested. Enriched food cultures were sampled after 18, 20, 22, 24 and 40 hours of enrichment for molecular assay testing. Samples were then evaluated using a qPCR method to quantify a molecular target representing a single gene copy/colony forming unit.

Results: After 18 hours of enrichment, 18 of 20 deli turkey samples were positive for *L. monocytogenes* (qPCR positive sample average: 1.5×10^6 copies/ml) while 20/20 were qPCR positive at 20 hours (qPCR positive average: 7.6×10^6 copies/ml) using the new single-step enrichment broth. 10/20 potato salad samples were found to be positive (qPCR positive average: 1.2×10^5 copies/ml) after 18 hours enrichment.

Significance: A new, single step *Listeria* enrichment broth was tested using fractionally-positive inoculated food samples. These enriched food samples were evaluated using a qPCR testing method targeting a single gene with 1 copy/cell to determine the density of cells present in the enriched food samples. Positive samples were found to contain at least 1×10^5 gene copies/ml in as short as 18 hours from the inoculated food samples. This enrichment medium was shown to be productive for the tested food samples in less than 50% of the time for standard enrichment methods using a single step format which provided densities detectable by most *Listeria* detection methods.

P2-16 Development of Real-time PCR Assays for the Molecular Detection of the Genes Encoding Flagellar Antigens H2, H8, H11 and H28

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Introduction: While *Escherichia coli* O157:H7 is the primary Shiga toxin-producing *E. coli* (STEC) serotype associated with foodborne illness outbreaks involving hemolytic uremic syndrome (HUS), several non-O157 STEC serotypes have also been implicated in HUS outbreaks in the United States, Europe, and Australia. Serotypes O145:H28, O111:H8, O103:H2, and O26:H11 are recognized worldwide among the most prevalent HUS-causing non-O157 serotypes.

Purpose: The purpose of this study was to develop real-time PCR assays to detect H antigen-encoding genes for H antigens commonly associated in emerging non-O157 STEC serotypes including H2, H8, H11, and H28.

Methods: Sequence data for the H antigen-encoding *fliC* gene was obtained from the NCBI GenBank database in order to generate consensus sequences for the H2 (5 sequences), H8 (11), H11 (5), and H28 (2) antigens using a sequence alignment software. For each H antigen, primer and probe sets for a 5′ nuclease assay were designed within *fliC*. Each assay was tested against a panel of 94 *E. coli* and non-*E. coli* isolates. H antigen assays were designed to work independently but also were optimized to be duplexed with an internal amplification control, as well as coupled to O antigen assays that had been previously designed and tested.

Results: The conserved and variable regions for the four H antigen gene targets were very closely related and provided a considerable challenge when designing the primer/probe sets. The H2, H8, H11, and H28 assays positively detected 5, 5, 4, and 2 strains as their respective H-types in the 94-isolate panel and showed no cross-reactivity with closely related serotypes. Interestingly, the H8 assay did not detect two isolates presumed to be H8 strains; subsequent classical serotyping confirmed the molecular serotyping result, further displaying the assay's specificity.

Significance: Non-O157 serotypes, such as O26:H11, are of major concern in HUS cases both in the U.S. and Europe, and development of molecular detection assays targeting specific antigens provides means to rapidly screen for STEC serotypes in foods.

P2-17 Optimization of a Real-time RT-PCR Assay Reveals an Increase of Genogroup I Norovirus in the Clinical Setting

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Introduction: There are approximately 23 million cases of norovirus illness reported each year, making noroviruses the most common cause of foodborne outbreaks and gastroenteritis in the U.S. While a large percentage of gastroenteritis outbreaks have been associated with norovirus infection, many suspect cases are not laboratory confirmed in stool specimens submitted for testing.

Purpose: This study was conducted to optimize a real-time RT-PCR assay for the improved detection of GI norovirus in patient specimens based upon sequence data from a collection of representative clinical norovirus sequences.

Methods: Ten previously determined GI-positive patient stool specimens were used to compare assay performance characteristics of the standard assay with the assay developed in this study. One hundred thirty-eight patient stool specimens collected from January 2010 to August 2010 were also tested with both assays. GI and GII noroviruses were not detected in these stool specimens by the standard assay used during routine diagnostic testing. Primers and probes for the optimized assay were designed manually from a representative alignment of 13 human clinical norovirus sequences that denote seven of the eight genotypes of Genogroup I.

Results: The optimized oligonucleotides demonstrated a 64-fold increase in sensitivity, a 2-log decrease in the limit of detection, and an 18% increase in amplification efficiency, when compared to the standard assay. The optimized test also detected GI norovirus in clinical specimens that were initially negative by the standard assay. Use of the optimized assay increased the annual positivity of GI norovirus from 1.2% to 4.5%, indicating the prevalence of GI norovirus may be higher than previously identified.

Significance: Laboratory confirmation of the etiologic agent involved in a gastroenteritis outbreak is of great value in public health remediation and prevention, as well as enhances our understanding of the prevalence and spread of norovirus in the U.S. population.

P2-18 Development of RNA Aptamers That Specifically Bind to *Escherichia coli* O157:H7

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Introduction: E. coli O157:H7 is one of the major pathogenic bacteria that are associated with the largest number of outbreaks related to the consumption of raw or under-cooked meats. Aptamers, which are artificial specific single stranded oligonucleotides, DNA or RNA, with the capability of binding specifically to non-nucleic acid target molecules, have been shown to be useful for diagnostic tools as alternative antibody.

Purpose: In this study, aptamers against *E. coli* O157:H7 were developed as a new diagnostic tool, and the sensitivity and specificity of the selected aptamers were evaluated.

Methods: A total of 10 rounds of systematic evolution of ligands by exponential enrichment (SELEX) using magnetic beads were applied to select RNA aptamers binding to *E. coli* O157:H7 from RNA aptamer library including random (N40) sequences. The selected aptamer pool at the end of 10 rounds of SELEX were then sequenced and labelled with fluorescein maleimide to 5′ of oligonucleotide. The fluoro-labeled aptamer binding assays using fluorescence microplate reader and flow cytometry were performed to determine the affinity, inclusivity and exclusivity of the aptamer candidates for specific detection of *E. coli* O157:H7. Strains of *E. coli* O157:H7 non-*E. coli* O157:H7, *E. coli*, and non-*E. coli* were used for the tests.

Results: The 10th round pool has higher affinity to *E. coli* O157:H7 than other round pools. The ten sequences with common motif were identified from the enriched RNA pool of the 10th round. The RNA aptamer with the highest fluorescence intensity, thus have the highest binding capacity to *E. coli* O157:H7, was selected. The selected aptamer was specifically bound to *E. coli* O157:H7 without cross-reactivity to non-*E. coli* O157:H7, *E. coli*, and non-*E. coli*.

Significance: The selected aptamer specific to *E. coli* O157:H7 has great potential for application to presumptive screening method or alternative serotyping method for *E. coli* O157:H7 in foods. Future work must focus on application of RNA aptamer to detect *E. coli* O157:H7 in food samples by aptasensor or aptamer-linked immobilized sorbent assay.

P2-19 Development and Evaluation of a Four-color Multiplex Real-time PCR Assay for *Salmonella* Detection in Tomatoes

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Introduction: Salmonella is the leading cause of foodborne illness in the United States, with an increasing number of reported *Salmonella* outbreaks originating from produce in recent years. Real-time PCR is an important rapid molecular detection tool that has great potential to minimize the risk of *Salmonella* outbreaks while also reducing time-to-result.

Purpose: We have developed a four-color real-time PCR assay that detects *Salmonella* species. The simultaneous combination of signal from *invA, apeE,* and *gapA* provides a unique signature for *Salmonella* detection.

Methods: The real-time PCR assay was designed against three *Salmonella* gene targets (*invA, apeE,* and *gapA*) as well as an internal positive control to monitor inhibition. The multiplex assay was developed using *Salmonella* reference collections (SAR-A, n=72; SAR-B, n=72; SAR-C, n=16; and SAFE, n=101). The assay was also evaluated along with sample preparation methods whereby 120 g tomato samples spiked with 1 to 10 CFU *Salmonella* Typhimurium were enriched 20 hr in mBPW and then prepared using (1) a pre-clarification tray and an automated magnetic bead-based method or (2) a manual clarification column method.

Results: The assay detected 261 out of 261 of the inclusivity *Salmonella* species isolates. All 20 non-*Salmonella* exclusion strains were not detected. The assay consistently detected 100 copies of *Salmonella* genomic DNA. When used in conjunction with sample preparation in a controlled tomato-spiking study, the assay detected 1 to 10 CFU *Salmonella* post-enrichment.

Significance: Our early evaluation demonstrates the high specificity and sensitivity of the assay when testing pure cultures and spiked tomato samples. We are in the process of evaluating the assay on environmental samples from the field. The results thus far show promise that this assay and a sample preparation workflow can be applied to *Salmonella* surveillance of field samples.

P2-20 Multi-laboratory Optimization and Evaluation of DNA Extraction Procedures for Repetitive Sequence-based PCR Characterization of Outbreak *Salmonella* Isolates

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Introduction: Repetitive sequence-based PCR (rep-PCR) has been shown to be applicable both for strain characterization and subspecies identification of microbial isolates. The DiversiLab system (bioMérieux Inc.) is a commercially available, semi-automated, rep-PCR based DNA fingerprinting system that has been shown to be less labor intensive than Pulsed Field Gel Electrophoresis (PFGE) and can provide results in <5 hours from a pure culture of bacteria. Although this system has been widely used in clinical settings to identify and distinguish microbial isolates at subspecies and strain levels, using the DiversiLab for foodborne pathogens is still in its infancy due to the lack of a comprehensive database of pathogens, specifically the outbreak strains.

Purpose: The objectives of this study are (i) to examine the performance of the DiversiLab system in data reproducibility and overall efficiency, and (ii) to optimize a semi-automated DNA extraction procedure with MagNA Pure Compact (Roche) along with the DiversiLab system for molecular typing.

Methods: Four outbreak *Salmonella enterica* isolates (two Typhimurium serotype and two Javiana serotype) were sent to 15 FDA /Microbiology Cooperative Agreement laboratories (MCAP) to perform DiversiLab analysis. The isolates were cultured on TSA + 5% sheep blood plates at 35 ± 2°C for 24 hours. DNA from each culture was extracted using both the MoBio manual kit (MoBio PowerLyzer UltraClean Microbial DNA Isolation kit) and a semi-automated (MPC Nucleic Acid Isolation Kit I), MPC method. The results from multiple labs were compared and analyzed for reproducibility and sensitivity of the DiversiLab system. To our knowledge, this is the first time the MPC method was optimized for use with the DiversiLab system to increase DNA extraction efficiency.

Results: Both manual DNA extraction and the MPC method generated highly reproducible and consistent rep-PCR DNA fingerprinting data among multiple labs (>10) with the DiversiLab System. The DiversiLab system discriminated *S. enterica* at the serotype level. No substantial differences on DNA banding patterns was found between the MPC and MoBio manual kit methods of extraction for the four *S. enterica* isolates tested.

Significance: This work represents the first step to develop a comprehensive foodborne pathogen database. It also shows that the DiversiLab can be used as an alternative tool for molecular typing and for tracking the sources of contamination in foodborne outbreaks.

P2-21 Molecular Assays for the Screening and Identification of Enteroinvasive *Escherichia coli* and *Shigella* Species

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Introduction: Shigellosis, or bacillary dysentery, is caused by the ingestion of as few as 10 to 200 of *Shigella* or 1 million enteroinvasive *Escherichia coli (EIEC)* organisms. Biotyping of EIEC and the four *Shigella* species: *S. flexneri*, *S. sonnei*, *S. boydii* and *S. dysenteriae* requires the use of antisera and takes several days.

Purpose: The project is the development of a multiplex PCR assay that will ultimately screen and tag EIEC and the four *Shigella* species with a barcode for their identification.

Methods: Alignments between the sequences available in public databases for EIEC and *Shigella* genomes highlight various regions exhibiting potential species specificity. Bacterial DNA lysates were prepared for 6 *E. coli* strains, 5 gram-negative non-enterobacterial strains, 58 EIEC isolates, 74 *S. flexneri* isolates, 56 *S. sonnei* isolates, 22 *S. boydii* isolates, 40 *S. dysenteriae* isolates, 27 uncharacterized *Shigella* isolates and 28 *Salmonella* isolates. These various DNA lysates were used in conventional PCR assays using primers covering presumably variable regions in the chromosome of EIEC/*Shigella*.

Results: The use of 24 primers covering 10 presumably variable chromosomal regions allowed us to generate 20, 11, 17, 8 and 12 different PCR amplification patterns for our collection of EIEC, *S. flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae*, respectively, creating a subgroup of 68 isolates for further analysis.

Significance: While earlier work by others indicated that *Shigella* and EIEC derived from ancestral commensal *E. coli* by convergent evolution, forming distinct subclusters that contain strains from mainly one serogroup, our initial analysis indicates a lot of variability amongst isolates belonging to EIEC or to the same *Shigella* species. The subgroup of 68 isolates is now being examined for the presence of species-specific signatures in 15 additional genomic regions.

P2-22 Sensitivity of Molecular-based Detection of *Shigella* in Produce

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Introduction: A highly sensitive molecular detection method is especially important for *Shigella*, considering that ingestion of as few as 10 to 200 of these bacterial pathogens can cause disease. Nevertheless, detection from food is particularly challenging due to the presence of the background microflora and of inhibitory organic compounds.

Purpose: The ORA produce assignment guidelines for *Shigella* use boiling in PBS as a standard DNA extraction method. The objective of this study was to compare the quality of the DNA lysates obtained from seven different DNA extraction methods on six different produce types.

Methods: The resident bacterial flora present on the surface of tomato, orange pepper, cucumber, green onion, parsley and cilantro were enriched overnight in *Shigella* broth plus Novobiocin following the ORA produce assignment guidelines. One ml of each culture [containing 1.7E9, 1.7E9, 1.7E10, 5.1E8 and 1.4E9 bacteria, respectively], were spiked with 5,000 copies of a plasmid pBluescriptKS+ or 14 or 6,700 of *S. flexneri* cells prior to DNA extraction and analysis by conventional PCR targeting pBluescriptKS+ or *Shigella* spp.

Results: While amplification of pBluescriptKS+ did not reveal the presence of PCR inhibitory compounds in any DNA lysates, only the Instagen DNA extraction method allowed the detection of *Shigella* DNA in the six commodities tested, when 6,700 *Shigella* cells were mixed with the resident flora. Although a new 3-plex PCR assay targeting *virB, mxiC* and *ipaH* virulence genes was used in this study, only *ipaH* was detected, in agreement with the multicopy presence on *Shigella* chromosome and virulence plasmid.

Significance: This study suggests that the Instagen DNA extraction method from Biorad increases the sensitivity of detection of *Shigella* in produce by conventional PCR, compared to boiling in PBS. Additional studies on Real-time and Digital PCR platforms will be attempted for confirmation.

P2-23 Evaluation of VIDAS® Easy *Salmonella* Assay (EasySLM) with ChromID™ *Salmonella* (SM2) Agar for the Detection of *Salmonella* in a Variety of Foods: Collaborative Study

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Introduction: The VIDAS® SLM assay is a specific enzyme-linked fluorescent immunoassay (ELFA) performed in the automated VIDAS instrument. The procedure is a 2-step enrichment procedure, using pre-enrichment followed by selective enrichment in a newly formulated broth, SX 2 broth.

Purpose: The purpose of this AOAC Collaborative Study was to compare the VIDAS Easy *Salmonella* method to the FDA BAM for vanilla ice cream, liquid egg, raw shrimp, spinach and peanut butter and the USDA-MLG method for deli turkey.

Methods: This new method was compared in a multi-laboratory, collaborative study to the FDA BAM Chapter 5 method for 5 food matrices (liquid egg, vanilla ice cream, spinach, raw shrimp and peanut butter) and the USDA MLG 4.04 method for deli turkey. Each food type was artificially contaminated with *Salmonella* at 3 inoculation levels. A total of 15 laboratories representing government, academia and industry, throughout the United States, participated.

Results: In this study, 1,583 samples were analyzed, of which 792 were paired replicates and 791 were unpaired replicates. Of the 792 paired replicates, 285 were positive by both the VIDAS and reference methods. Of the 791 unpaired replicates, 341 were positive by the ELFA method and 325 were positive by the cultural reference method. A Chi-square analysis of each of the 6 food types, at the 3 inoculation levels tested, was performed and showed no statistically significant differences between the ELFA method and the reference methods.

Significance: For all foods evaluated, the VIDAS Easy SLM method with ChromIDSM2 Agar demonstrated comparable results to the reference methods for the rapid, automated detection of *Salmonella*.

P2-24 Differentiation of Epidemic Clones III and IV of *Listeria monocytogenes* Using Fourier Transform Infrared (FTIR) Spectroscopy and Multivariate Analysis

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Introduction: Rapid, non-invasive techniques for testing food samples to detect pathogenic *Listeria monocytogenes* are needed. Fourier transform infrared (FTIR) micro-spectroscopy is a cutting-edge technique that can be used to generate biochemical fingerprints for differentiation and identification of different bacterial strains.

Purpose: The objective was to determine if isolates of *L. monocytogenes* epidemic clones (EC III and IV; strain numbers: J1-101, J1-129, J1-220 and R2-499) could be differentiated using FTIR micro-spectroscopy and multivariate analysis.

Methods: Each of the epidemic clones was grown individually in trypticase soy broth yeast extract at 37°C for 18 to 24 hours, aliquots were centrifuged, the pellet washed three times and suspended in 100 µl PBS for infrared [IR] slide preparation or for enumeration on Petri-film. Ten µl from each of the final epidemic clone suspension in PBS was transferred onto coded IR slides and dried for about 2 hours in a fume chamber. An IR microscope (Bruker Hyperion-3000) with Tensor T27 FTIR spectrometer was used for spectral readings from the slides, and discrimination of epidemic clones of *L. monocytogenes* was done using algorithms based on multivariate statistics.

Results: The microbial count on the Petri-film for each epidemic clones loaded onto the IR coded slides was approximately 10^7 cells per ml. The Mahalanobis maximum within group distances for strains J1-101, J1-129, J1-220 and R2-499 were ~ 1.20 x 10^6, ~ 1.79 x 10^7, ~1.45 x 10^7 and ~1.15 x 10^7, respectively. The minimum across group distances obtained by comparing the different epidemic clones generated different numbers and there was 100% success in the differentiation analysis.

Significance: This finding indicates FTIR can be used for rapid differentiation analysis of variants of *L. monocytogenes*. This technology will be of great importance in surveillance of rapidly mutating and emerging pathogens.

P2-25 Developing a *Staphylococcus aureus* Enterotoxin-specific Antibody for Establishing an Immunoquantitative Detection System

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Introduction: Staphylococcal food poisoning (SFP) is one of the most common foodborne diseases worldwide mainly resulting from the ingestion of *Staphylococcus aureus* enterotoxin (SE) produced by enterotoxigenic *S. aureus* strains. Because not all *S. aureus* strains are enterotoxigenic, SFP diagnosis should be based on the detection of SE in food. The sensitivity of detection limits of SE detection systems are needed to be improved to establish the intoxication dose for use with scientific risk analyses.

Purpose: The purpose of this study was to develop SEA, SEC, SEE, SEH-specific antibody and to establish a quantitative detection system using the immunoquantitative PCR (iqPCR) technology.

Methods: S. aureus FRI913, FRI137 and FRI569 strains were used for amplification of *sea, sec, see* and *seh* genes. Oligonucleotide primers were designed to amplify the mature forms of SE toxins excepting signal peptides and the amplicons were cloned into His6-tagging pET system to be expressed. Expressed toxins were used for immunizing mice to produce monoclonal antibodies and five different sandwich formats consisting of capture and detection antibodies were tested with ELISA. To increase the sensitivity, 5′ biotinylated reporter dsDNA were bound to the biotinylated detection antibody via streptavidin. PCR amplification targeting the reporter dsDNA was monitored using SYBR green. SE production was investigated in agricultural food products using the developed systems.

Results: Several ELISA formats were chosen based on their sensitivity, thereby offering a more robust reactivity profile compared to other formats. Detection of SE with iqPCR was started at about a Ct greater than 20, allowing quantitative determination in the linear Ct range from approximately 23 to 30. Average measurements obtained with iqPCR with standards SE spiked in food and laboratory media samples showed that the sensitivity was approximately 1,000 times higher than the commercial ELISA systems. iqPCR analysis of agricultural food products showed very low prevalence and quantity of SE in foods.

Significance: In many SFP, *S. aureus* cells were not isolated, suggesting that there is a need for reconsideration of conventional analyses, which focus mainly on detection of *S. aureus*. This study contributed to develop more sensitive methods of SE detection for establishing intoxication dose and the scientific risk analysis by governments and research institutes.

P2-26 A Comparison Study of the VIDAS® *Listeria monocytogenes* Xpress (LMX) to the USDA-FSIS and Health Canada MFHPB-30 Methods for the Specific Detection of *Listeria monocytogenes* in Ready-to-Eat Meats

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Introduction: The VIDAS® *Listeria monocytogenes* Xpress (LMX) test is an enzyme-linked fluorescent immunoassay (ELFA) designed for use with the automated VIDAS® or mini-VIDAS® instruments for the specific detection of *Listeria monocytogenes* using a 26-hour proprietary enrichment broth.

Purpose: As part of the AOAC® Research Institute GovVal program, the ELFA test method was compared to the Health Canada MFHPB-30 and USDA methods for the detection of *Listeria monocytogenes* in ready-to-eat meats. The alternative method also included the use of the chromogenic media, chromID™ Ottaviani Agosti Agar (OAA) and chromID™ Lmono, for confirmation of LMX presumptive results.

Methods: Four ready-to-eat meats (hot dogs, deli turkey, deli ham, and liver paté) were each inoculated with a different *Listeria monocytogenes* strain at two levels and, along with uninoculated test portions, were analyzed by each method. Twenty replicates of each inoculation level and five uninoculated controls were evaluated. To determine if the methods were equivalent, a Mantel-Haenszel Chi-square for unmatched test portions was calculated for each test method, reference method combination.

Results: For all foods tested, there was no significant difference in the Chi-square value for the test method when compared to both the USDA and Health Canada reference methods. There were 10^8 total positives for the LMX method and 10^7 positives for both the USDA and Health Canada methods. Confirmation of presumptive LMX results with the chromogenic OAA and Lmono media was shown to be equivalent to the appropriate reference method agars.

Significance: These data indicate that the VIDAS LMX assay is an acceptable alternative method for the detection of *Listeria monocytogenes* in foods that provides next-day results compared to up to 5 days for the reference method.

P2-27 Validation Study of the Reveal® *Listeria* 2.0 Method for Detection of *Listeria* spp. in Foods and Environmental Samples

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Introduction: Reveal® *Listeria* 2.0 is a new lateral flow immunoassay for detection of *Listeria* spp. in foods and environmental samples. Following single-step enrichment for 27–30 hours, an aliquot of the enrichment is heat killed and tested using the test device. Samples producing positive assay results are confirmed using standard culture methods.

Purpose: The purpose of the study was to compare performance of the lateral flow method with that of the USDA/MLG and FDA/BAM reference culture procedures for detection of *Listeria* spp. in a variety of inoculated foods and sponge or swab samples from environmental surfaces.

Methods: Inclusivity and exclusivity testing was conducted with pure cultures grown in LESS broth and tryptic soy broth, respectively. Foods tested included dairy and egg products, processed meats, and processed seafoods. Environmental surfaces tested included stainless steel, plastic, ceramic, and concrete. Samples for testing with the lateral flow method were enriched in LESS broth for 27–30 hours at 30°C. Samples for testing by the USDA/MLG or FDA/BAM procedures were enriched in accordance with the published methods. In each trial, twenty inoculated and five control samples were prepared for each method. Results were analyzed using the Mantel-Haenszel Chi-square test for unpaired sample data.

Results: Results of inclusivity testing showed that the lateral flow test detects all species of *Listeria* except *L. grayi*. Exclusivity panel strains all tested negative or did not grow in LESS broth. Of 10 foods tested, there were no significant differences in the number of positive results obtained with the lateral flow and reference culture methods as determined by Chi-square analysis ($P < 0.05$). For foods tested in comparison to the FDA/BAM method, relative sensitivity of the lateral flow method was 98.1%. For foods tested in comparison to the USDA/MLG method, relative sensitivity of the lateral flow method was 100%. For environmental samples, two surface types (plastic and sealed concrete) yielded significantly more positives by the test method in comparison to the USDA/MLG procedure. For stainless steel and ceramic, results of the test and reference method analyses were not statistically different. Relative sensitivity of the lateral flow method in comparison to the USDA/MLG method for environmental samples was 127%. There were no unconfirmed positive results by the lateral flow assay in any phase of the study.

Significance: Results of this study showed that the Reveal *Listeria* 2.0 method is an effective procedure for detection of *Listeria* spp. in a variety of foods and environmental samples, with high specificity and sensitivity comparable to that of the reference procedures.

P2-28 Evaluation of Commercial Antibodies against *Escherichia coli* O157:H7 for Development of Improved and Sensitive ELISA-based Detection Systems

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Introduction: E. coli O157:H7 has been recognized by the Centers for Disease Control and Prevention (CDC) as one of the major pathogens implicated in severe foodborne related illness that results in death. It is estimated to cause not only thousands of illness cases, but hundreds of fatalities per year in the United States alone. In addition, this pathogen results in product recalls totaling millions of dollars in losses. Therefore, it remains critical to develop a rapid, robust, sensitive, and user-friendly portable assay for detection in food matrices.

Purpose: The purpose of this study was to evaluate commercially available antibodies against *E. coli* O157:H7 in an ELISA-based detection system with downstream application for improved, sensitive, and rapid detection in foods.

Methods: A standard sandwich ELISA was developed utilizing commercially available antibodies to whole *E. coli* O157:H7 cells. Bound peroxidase-labeled antibody was detected by absorbance at 450 nM using an ultrasensitive 3,3',5,5'-tetramethylbenzidine solution. Ten-fold serial dilutions of *E. coli* O157:H7 isolates (n=7) and non-O157:H7 isolates (n=7) were evaluated in triplicate. The detection limit of the assay was determined.

Results: This ELISA assay detected 6 out of the 7 *E. coli* O157:H7 isolates with detection limits at 5.03×10^4 Colony Forming Units (CFU)/ml. One *E. coli* O157:H7 isolate was not detected. Four of the 7 non-O157:H7 isolates were also detected with detection limits ranging from 5.29×10^5 to 7.57×10^8 CFU/ml.

Significance: These data suggest that commercially available antibodies can detect *E. coli* O157:H7, but are also cross-reactive with other *E. coli* non-O157 strains. Further evaluation and development of this assay is required to improve detection sensitivity and specificity. This will require antibodies for utilization in specific ELISA-based *E. coli* O157:H7 detection systems that can discriminate between O157 and non-O157 strains of *E. coli*.

P2-29 The Evaluation of a VIDAS Next-day Method for Detection of *Salmonella* in Food

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Introduction: A new immunoassay, the VIDAS UP *Salmonella* (VIDAS SPT) for detection of *Salmonella* in food has been developed by bioMérieux (Marcy L'Etoile, France). This assay incorporates phage proteins to increase the sensitivity and specificity compared to traditional immunoassays.

Purpose: The study was undertaken at Silliker Australia, to evaluate the sensitivity, specificity and convenience of this method for detection of *Salmonella* in foods.

Methods: In the new method, samples are enriched overnight in Buffered Peptone Water (BPW) with the addition of a selective supplement. Samples may be tested in the VIDAS instrument after BPW enrichment or transferred to a selective broth SX2 and incubated for a further 4 hours. Eight foods representing raw foods with a high microbial load or processed foods with stressed inoculum were chosen to challenge the method. These foods were inoculated with *Salmonella* at low levels (target 1–5 CFU/25 g). Ten inoculated and one uninoculated replicates were compared to standard reference methods (USDA, FDA, BAM). In addition, 200 uninoculated samples, representing a variety of foods submitted for routine testing, were analyzed.

Results: The next-day method gave comparable results to reference culture methods. For some foods a 4 h incubation in SX2 broth gave improved recovery or facilitated confirmation. For the 200 uninoculated samples, 199 gave negative results, and one sample giving a positive result was confirmed positive by subculture.

Significance: The VIDAS SPT assay was sensitive and specific. This represents a significant advance in *Salmonella* testing, allowing a presumptive result within 24 hours after a simple enrichment procedure.

P2-30 Preliminary Evaluation of VIDAS® UP *Salmonella* (SPT) Assay for the Next-day Detection of *Salmonella* in 375 g Samples of Select Food Types

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Introduction: The detection of *Salmonella* in large food samples (375 g) without compromising the sensitivity and rapidity could help to enforce food safety objectives and increase the probability of finding low levels of contamination. An automated phage protein ligand assay with the capability of next-day detection of *Salmonella* in a more representative sample size could be a promising solution. This new generation VIDAS® UP *Salmonella* (SPT) assay uses a novel combination of recombinant phage proteins and monoclonal antibodies to target both somatic and flagellar antigens for the detection of motile and non-motile strains of *Salmonella*.

Purpose: The purpose of this study was to perform a preliminary evaluation of the phage protein ligand assay for next day detection of low levels of *Salmonella* (1-5 CFU/ sample) in 375 g samples of dark chocolate, lettuce and dried dog food with comparison to the FDA-BAM reference method.

Methods: Ten artificially contaminated 375 g food samples of each matrix tested (dried dog food, lettuce and dark chocolate) were diluted (1:4) with pre-warmed buffered peptone water containing 5 ml of a specific supplement. Samples were incubated at 42°C ± 1.0°C for 18 and 20 h. Additionally, after 18 h of incubation, 1 ml of each sample was transferred to 10 ml pre-warmed SX2 broth and further incubated for 4 h. The enriched samples from all three different incubations were heated (95°C ± 5°C) and then cooled followed by analysis using phage protein ligand assay. All samples were simultaneously plated on XLD and/or Chrom ID *Salmonella* agar and confirmed using API 20 E strips. The reference method (FDA-BAM) was performed in parallel for all samples. Uninoculated samples of each food type were evaluated as negative controls.

Results: The results for 10 inoculated and 1 uninoculated sample obtained for the phage protein ligand assay to detect *Salmonella* in all food matrices were not statistically significantly different when compared with reference method using unpaired chi-square analysis at 5% level of significance.

Significance: The evaluation of phage protein ligand assay provided satisfactory results for the detection of *Salmonella* in 375 g artificially contaminated samples and uninoculated controls. This new generation of assay could provide an automated solution for next day detection of low contamination levels of *Salmonella* in larger sample sizes.

P2-31 Evaluation of Chromogenic Media for Isolation and Detection of *Salmonella* spp. from Foods

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Introduction: Salmonella is the leading cause of foodborne bacterial disease outbreaks. Isolation and detection of *Salmonella* from foods remains a big challenge. Lack of effective media and enrichment broths is a major limiting factor.

Purpose: The objective of this project is to evaluate the effectiveness of chromogenic media for detection and isolation of *Salmonella* from foods.

Methods: Xylose lysine deoxycholate agar, Hektoen enteric agar, and Bismuth sulfite agar were conventional selective media used as control. BIOLOG Rainbow agar *Salmonella*, BIO-RAD RAPID *Salmonella*, CHROMagar™ *Salmonella* Plus, HardyCHROM *Salmonella*, Brilliance *Salmonella* agar and R&F *Salmonella* (nontyphoidal) chromogenic plating medium were selective chromogenic culture media selected for the study. Seventy-eight *Salmonella* isolates, including all the subspecies, and 24 non-*Salmonella* isolates, including 15 species, were used for inclusivity and exclusivity tests of all the media. Tomato, pepper and lettuce were inoculated with 5 *Salmonella* isolates individually at 1 to 10 CFU/25 g food. Enrichment samples were streaked on all the media to evaluate the effectiveness of the chromogenic media in comparison with the conventional media.

Results: No typical *Salmonella* colonies were observed on any of the 9 conventional and chromogenic media when the 25 non-*Salmonella* isolates were streaked on these media. The exclusivity of the tested chromogenic media was equivalent to that of the conventional selective media. Preliminary results indicated that BIOLOG Rainbow agar *Salmonella*, BIO-RAD RAPID *Salmonella* and HardyCHROM *Salmonella* had slightly better inclusivity than the other chromogenic and conventional media.

Significance: Currently, chromogenic media are not as widely used as conventional selective media. This research showed that some chromogenic media, such as HardyCHROM *Salmonella*, BIOLOG Rainbow agar *Salmonella*, and BIO-RAD RAPID *Salmonella*, are equivalent or better than conventional media for detection of *Salmonella*. Effective chromogenic media could improve the detection of *Salmonella*.

P2-32 Quantitative Evaluation of Three Selective Media for *Bacillus cereus* in Various Foods

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Introduction: Bacillus cereus (*B. cereus*) has been increasingly recognized as the causative agent of food poisoning outbreaks. The direct plating on selective media has been used for standard quantitative evaluation method of *B. cereus* in food samples.

Purpose: In this study, three different selective media: mannitol yolk polymyxinB agar (MYPA), polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA), and brilliance *Bacillus cereus* agar (BBCA) were compared for their recovery effectiveness and selectivity in artificially contaminated foods.

Methods: Three types of ready-to-eat (RTE) foods (triangle Kimbab, instant pumpkin soup, and radish sprout), powdered infant formula (PIF) and Korean traditional fermentation food (Soy bean paste) purchased from a local retail market in Seoul were used. To determine the effect of background microflora on the detection of *B. cereus*, a mesophilic aerobic plate count was performed in uninoculated food samples using plate count methods. For artificial inoculation, *B. cereus* was spiked into 25 g of food samples. Each sample was suspended in 225 ml of Butterfield's phosphate-buffered water followed by homogenization for 2 min with stomacher. After stomaching, 0.1 ml of homogenate was serially diluted (10-fold) in saline water and each dilution was inoculated on three selective media. Plates were incubated at 30°C (MYPA) or 37°C (PEMBA and BBCA) for 24 h. Plates that contain estimated 15-150 suspicious colonies were selected and five of typical colonies on each plate were sub-cultured. Presumptive positives were finally confirmed with real-time colony PCR. Colonies were counted and the extent of inoculation was estimated. All experiments were repeated three times for statistical analysis.

Results: The recovery effectiveness was higher ($P < 0.05$) on BBCA than on MYPA or PEMBA especially in foods with high background microflora such as radish sprout and soy bean paste. The competing flora were also much less on BBCA in foods with high background microflora so that differentiation of suspected colonies was easier on BBCA compared to other selective media.

Significance: It appears that BBCA, a chromogenic agar, could be a useful option for its good recovery effectiveness and selectivity especially in foods with a high level of background microflora such as fermented foods or fresh vegetables.

P2-33 Validation of the Soleris® *Escherichia coli* Method for Detection and Semi-quantitative Determination of *E. coli* in Foods

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Introduction: Food companies and testing organizations are seeking validated tools to help safeguard the public from organisms such as *Escherichia coli*. The Soleris® *E. coli* Medium vial in conjunction with the Soleris instrument system is a rapid and automated diagnostic tool for real-time detection of *E. coli* in a variety of food products. The instrument utilizes temperature-controlled incubation chambers and a photodiode-based optical detection device for measurement of color changes in the vial, caused by microbial growth.

Purpose: A study was conducted to compare the performance of the Soleris *E. coli* method against the ISO 7251 (Third edition 2005-02-01) Most Probable Number (MPN) reference method for detection of generic *E. coli* in food matrices.

Methods: Six generic *E. coli* strains originating from food sources were spiked into six food commodities: frozen green beans, echinacea powder, cocoa powder, sweetened condensed milk, pasteurized liquid egg and shredded mozzarella cheese. A dilution of the test sample homogenate was inoculated directly into the Soleris *E. coli* Medium vial. The test was used in a "dilute-to-specification" or threshold manner, in which the result is positive or negative above or below a desired cutoff (in colony forming units/gram (CFU/g) determined by the dilution and volume of sample homogenate added to the vial. The protocol assumes that 1 CFU introduced into the *E. coli* vial will lead to a positive result. The test was also used for "zero tolerance" determinations (e.g., absence in 25 g) by performing an off-line pre-enrichment step and then transferring a portion of the pre-enrichment culture to the *E. coli* vial. Uninoculated control samples were included in each trial.

Results: The results obtained by both the Soleris *E. coli* Medium vial system and the ISO 7251 MPN method were shown to be in agreement with 95% confidence, when determining the absence of *E. coli* in 25 grams of sample using the Mantel-Haenszel Chi-Square analysis. Results from both the Soleris *E. coli* Medium vial for the Dilute-to-Specification procedure and the ISO 7251 MPN method were found to be in agreement as determined by a statistical probability of detection analysis.

Significance: Results of these studies demonstrate that the real-time *E. coli* method can be successfully utilized in place of the ISO 7251 MPN method with a time savings of 2 to 3 days.

P2-34 Development of Non-O157 Shiga Toxin-producing *Escherichia coli* STEC O-type Specific Antibodies and Their Application for the Analysis of Raw Beef

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Introduction: E. coli O157:H7 is the most common Shiga toxin-producing *E. coli* (STEC) associated with human illness in the U.S. and is regulated by the USDA FSIS as an adulterant in raw beef. Recently, the USDA has proposed monitoring other non-O157 STEC linked to human illness and is initially targeting serogroups O26, O45, O103, O111, O121, and O145. The development and implementation of rapid methods for the analysis of these STEC in raw beef products is essential in order for industry to meet these new proposed food safety standards.

Purpose: The purpose of this study was to develop serogroup-specific antibodies against the USDA top six non-O157 STEC and apply these to the analysis of raw beef.

Methods: Polyclonal antibodies were developed against the top six non-O157 STEC serogroups. The antibodies were incorporated into lateral flow test strips and tested against a panel of 29 STECs and 34 non-STEC bacteria from 11 genera. Rabbit antibody-based test strips were applied to the analysis of raw ground beef. Positive samples were subjected to immunomagnetic separation (IMS) followed by cultural and biochemical confirmation.

Results: In control experiments, the antibodies showed 100% sensitivity and 100% specificity. Application of these antibodies for testing raw beef, showed that over 50% of the samples were positive for at least one of the non-O157 STEC serogroups. Serogroups O45 and O103 were the most prevalent in the samples tested. *E. coli* O45 and *E. coli* O145 isolates were found in addition to *Enterobacter cloacae* (O103) indicating that some strains of this bacteria share similar serology as *E. coli* O103.

Significance: The use of highly-specific antibodies for the isolation and/or detection of STEC in raw beef should save time and reduce cost of analysis. The antibodies could be used as IMS reagents or incorporated into rapid assays for sample screening prior to cultural confirmation. Our initial application of these antibodies suggested a relatively high prevalence of some non-O157 STEC serogroups.

P2-35 Determination of Antimicrobial Activity of Sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157:H7 Isolated from Various Samples

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Introduction: Many naturally occurring compounds found in medicinal plants, herbs and spices have been shown to possess antimicrobial activities against many foodborne pathogens. The use of these plants as a natural antimicrobial agent is gaining popularity. Sorrel (*Hibiscus*

sabdariffa) is widely used for the treatment of diseases. *Escherichia coli* O157:H7 has been recognized as a significant cause of foodborne and waterborne illness in the industrialized world.

Purpose: The objective of this study was to investigate the antimicrobial activity of Sorrel on *Escherichia coli* (*E. coli*) O157:H7 isolates from food, veterinary and clinical samples.

Methods: Phenolics of the calyces were extracted from 10 g ground, freeze-dried samples using 100 ml of 80% aqueous methanol. Concentrations of 10, 5 and 2.5% methanol extract of Sorrel were investigated for its antimicrobial activity. Inhibition zones were indicated by a lack of microbial growth due to inhibitory concentrations of Sorrel diffused into semisolid culture media beneath the Sorrel-impregnated disk.

Results: The results of this experiment showed that the most potent Sorrel concentration was the 10, 5 then 2.5%. The overall mean zone of inhibition for the Sorrel extract of 10% was 12.66 mm, 5% was 10.75 and for the 2.5% was 8.9 mm. The highest inhibition zones (11.16 mm) in veterinary and the lowest (10.57 mm) in the food samples were observed. There were significant ($P < 0.05$) differences between mean zones of inhibition displayed in the food, veterinary and clinical sources. Based on source of samples and concentration of Sorrel extract, the lowest mean inhibition was 7.00 ± 0.04 mm from clinical and the highest was 15.37 ± 0.61 mm from food source.

Significance: It indicated that Sorrel was effective at all levels in inhibiting *Escherichia coli* O157:H7, thus it possesses antimicrobial activity and holds great promise as an antimicrobial agent.

P2-36 Inhibition of *Escherichia coli* O157:H7 Beef Product Isolates by Cold-pressed, Terpeneless Valencia Orange Oil at Various Temperatures

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Introduction: Naturally occurring plant essential oils have been shown previously to exhibit antimicrobial activities against various microorganisms.

Purpose: This study was undertaken in order to identify an antimicrobial for potential effectiveness during chilling in beef processing, including periods of temperature abuse.

Methods: Unique to this study, cold-pressed, terpeneless Valencia orange oil was examined in conjunction with three temperatures (37°C, 10°C and 4°C) to determine its antimicrobial activity against three strains of *Escherichia coli* O157:H7 recovered from beef products, *E. coli* 933, 505B and ATCC 43895. The strains of *E. coli* were tested using a ninety-six well microtiter plate method with TSB+0.15% agar and a growth indicator, 2, 3, 5-triphenyl tetrazolium chloride (TTC) as the medium. Serial two-fold dilutions of cold-pressed, terpeneless Valencia orange oil were used, resulting in concentrations of oil ranging from 25 to 0.2% or 10 to 0.1%. Plates were incubated statically at 37, 10, or 4°C, and sampled at specific time intervals.

Results: After 6 hours at 37°C, all strains were inhibited at concentrations ranging from 0.6% to 0.2%, with a mean of 0.4 ± 0.01%. At 10°C, all strains were shown to be inhibited at concentrations ranging from 6.3 to 0.8%, with a mean of 1.1 ± 0.2%, after 6 hours. At 4°C, all strains were shown to be inhibited after 6 hours at concentrations ranging from 4.6 to 2.3%, with a mean of 3.5 ± 2.1%. After 24 hours at 4°C the strains were inhibited at concentrations ranging from 1.0% to 0.7% with a mean of 0.8 ± 0.3%. The ranges appear to be the result of effects from the variable nature of a complex media and an antimicrobial that presents potential multiple mechanisms for inhibition.

Significance: It appears cold-pressed, terpeneless Valencia orange oil is a viable option to inhibit *E. coli* O157:H7 at refrigeration temperatures.

P2-37 Antimicrobial Activity of Xoconostle (*Opuntia matudae) against Escherichia coli* O157:H7

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Introduction: Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit foodborne bacteria and extend the shelf life of processed food. Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against food pathogens. Xoconostle (*Opuntia matudae*) has recently attracted the food industry attention. Xoconostle has demonstrated strong anticancer and antioxidant characteristics due to the phenolic compounds content.

Purpose: This study focuses on the significant antibacterial activity of xoconostle pears against *Escherichia coli* O157:H7 in culture medium BHI.

Methods: Four strains of *Escherichia coli* O157:H7 with approximate populations of 3 logs CFU/ml were individually inoculated into BHI broth samples containing different concentrations of xoconostle extract. Samples were individually incubated at 37°C for 8 hrs. During the incubation period, bacterial growth was determined (turbidity via optical density at 610 nm) at 2 hrs intervals. At the end of the incubation period, the BHI broth was also diluted in sterile 0.05% peptone water and then surface plated onto BHI agar. Agar well diffusion assay was used to determine the minimum inhibitory volume (MIV) (the lowest volume that inhibits growth) and the minimum lethal inhibition volume (MLV) (the lowest volume that shows significant growth inhibition within three days of incubation).

Results: Results showed that bacterial population in control reached 8–9 logs CFU/ml, while the addition of 10% xoconostle extract caused the bacterial population to remain within 3–4 logs CFU/ml. The minimum inhibitory volume (MIV) was 275 µl/ml (V/V) and the minimum lethal inhibition volume (MLV) was 550µl/ml (V/V).

Significance: These results indicated that xoconostle has potential antibacterial effects against the growth of *Escherichia coli* O157:H7. Xoconostle can be an effective substitute to artificial antibacterial and can mitigate food safety risks.

P2-38 Survival of *Escherichia coli* O157:H7 in Topographical Features on Stainless Steel Surfaces from Gas and Liquid Phase Chemical Treatments

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Introduction: Examining factors such as fluid dynamics and topographical features which form microenvironments and promote pathogen survival will allow for improved treatments to effectively eliminate pathogens and thus reduce the risk of recontamination. This study evaluated the effect of microenvironments on stainless steel surfaces on the efficacy of antimicrobial agents for treating biofilms.

Purpose: To identify microenvironments that harbor and potentially protect pathogens from treatments and, in turn, become a source of microbial recontamination.

Methods: Eight-hour *E. coli* O157:H7 biofilms formed on stainless steel test surfaces with and without fabricated grooves (<100–500 µm wide and 100–750 µm deep) using a flow-through system were treated with antimicrobial agents hypochlorite (20–2000 ppm) and chlorine dioxide (0.7–2.2 mg l-1) for 5 –30 min. An isolation system was developed for two growth-based assays, recovery and regrowth, to determine efficacy of treatment. COMSOL Multiphysics was used to model flow and diffusion of antimicrobial agents in tested microenvironments.

Results: Results indicated that deeper grooves afforded cells more protection against antimicrobial agents. Although the recovery assay showed 200 ppm hypochlorite for 5 min led to a 6 log reduction on all surfaces, the regrowth assay indicated 30% of the samples survived treatment at deeper grooves. Diffusion models showed that 10 min was needed for maximum treatment concentration to reach the bottom of the deeper grooves. Treatment with chlorine dioxide gas at 0.7 mg l-1 (236 ppm) for 10 min also was not successful in eradicating the biofilm; however, chlorine dioxide concentration of 2.2 mg l-1 (741 ppm) for 5 min eliminated regrowth. Microenvironments of narrower grooves have been fabricated and are currently being tested.

Significance: Our findings will identify problematic scenarios (where pathogens are most difficult to kill) that should be evaluated when optimizing treatment regimens to effectively eliminate pathogens on food contact surfaces.

P2-39 Inactivation of *Alicyclobacillus acidoterrestris* with High Pressure Homogenization and Dimethyl Dicarbonate

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Introduction: Alicyclobacillus acidoterrestris is a sporeforming bacterium that can cause spoilage in fruit juices. This bacterium, more specifically its spore, is problematic to the juice industry because of its ability to grow in low pH environments and survive pasteurization processes.

Purpose: The purpose of this study was to investigate the effect of non-thermal technology (high pressure homogenization (HPH) and the antimicrobial compound, dimethyl dicarbonate (DMDC), on inactivation of *A. acidoterrestris* in a broth system.

Methods: Vegetative cells and spores of five strains of *A. acidoterrestris* were screened for their sensitivity to HPH (0, 100, 200 and 300 MPa) in *Bacillus acidoterrestris* thermophilic (BAT) broth. The strain most resistant to HPH (strain SAC from apple juice concentrate) was further tested for sensitivity to DMDC (0 and 250 ppm). This was followed by evaluation of combined effects of HPH and DMDC against the strain. Effects of HPH and DMDC treatment combinations (250 ppm DMDC added 2 h before, immediately before, and immediately after 300 MPa HPH treatment) on spores of SAC over a 24-h period were evaluated. After all treatments, samples were serially diluted and surface plated onto BAT agar, and populations determined after incubation at 44°C for 48 h.

Results: All treatments significantly ($P < 0.05$) inhibited growth of vegetative cells whereas spores were less affected. HPH caused a 1–2 log reduction in initial vegetative cell populations at 300 MPa for four strains, but only about 0.5 log reduction of the SAC strain. For all five strains, there was < 0.5 log reduction of spore populations. DMDC also slowed growth of vegetative cells significantly. For vegetative cells of SAC, 24 h of incubation in broth with 250 ppm DMDC was required to reach 7 log CFU/ml, while only 10 h was required to reach the same population without DMDC. However, only a 2-h difference in time to reach 7 log CFU/ml was required when spores were cultured with (12 h) or without DMDC (10 h). The addition of DMDC, together with HPH, did not increase inhibition of growth of *A. acidoterrestris*.

Significance: These results demonstrate that HPH and DMDC show promise for aiding in control of growth of vegetative cells of *A. acidoterrestris*. However, neither treatment, alone or in combination, is very effective against spores.

P2-40 Antibotulinal Activity of Sodium Salt Replacers in Laboratory Media

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Introduction: Sodium salts are used in processed foods as an integral factor in ensuring microbial safety either by reducing water activity or by direct antimicrobial activity. It has been proposed that the total sodium content in these foods be reduced by partially replacing sodium salts with potassium salts or low concentrations of antimicrobials. There is limited data directly comparing these treatments with their sodium counterparts' ability to inhibit growth of *Clostridium botulinum*.

Purpose: This project compares the inhibitory effect of potassium or calcium alternatives to sodium chloride, lactate, benzoate and propionate against *C. botulinum*.

Methods: Trypticase-Peptone-Glucose-Yeast Extract (TPGY) media was supplemented with sodium or non-sodium salts, pH adjusted to 6.2, inoculated with 3-log CFU/ml of proteolytic *C. botulinum* spores and heat shocked at 74°C. Salt treatments included sodium and potassium chloride, sodium and potassium lactate (1.5, 3.0, 4.5%); sodium and potassium benzoate, sodium and calcium propionate (0.1, 0.2, 0.3%), sodium nitrite (156 ppm) and no antimicrobial controls. Five tubes per treatment and an uninoculated treatment tube were incubated anaerobically at 15 and 25°C and optical density (absorbance at 600 nm) measured every 2-3 days, through 28 days. The study was replicated twice.

Results: As expected, growth of *Clostridium botulinum* was slower at 15°C than at 25°C, though growth trends among the various antimicrobial treatments were similar for both temperatures. Growth (OD > 0.1) was observed on days 2 and 7 for No Antimicrobial Control treatments stored at 25 and 15°C, respectively, whereas no growth was observed throughout the 28 day testing interval for TPGY with 156 ppm $NaNO_2$. The greatest difference between the various salts was seen at the lowest antimicrobial level (0.1% benzoate or propionate or 1.5% chloride or lactate) at 15°C. Both 0.1% calcium and 0.1% sodium propionate supported *C. botulinum* growth at Day 7 whereas 0.1% potassium benzoate delayed growth until 14 days, and no growth was detected at 28 days for 0.1% sodium benzoate. Growth was delayed until days 9, 11, 11, and 14 for 1.5% KCl, NaCl, K-lactate and Na-lactate, respectively.

Significance: These results show that sodium-based versions of propionate, benzoate, lactate and chloride are equivalent or more effective than their non-sodium counterparts for inhibiting *C. botulinum*, when used on a percentage basis.

P2-41 Inhibition of *Colletotrichum gloeosporioides* by Mexican Oregano Essential Oils Added to Edible Films

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Introduction: Colletotrichum gloeosporioides is the causal agent of anthracnose, an important spoilage mold in tropical fruits. Recently, fungal chemical resistance has been developed because of the use of higher concentrations of such products, increasing the risk of high levels of toxic residues inside the fruit. The use of plant extracts is believed to be safer to consumers as well as the environment. In these sense, edible films can incorporate these extracts to provide microbiological stability and reduce the risk of microbial growth on food surfaces.

Purpose: The aim of this study was to evaluate the inhibition of *Colletotrichum gloeosporioides* by selected concentrations of Mexican oregano (*Lippia berlandieri* Schauer) essential oil added to chitosan or starch edible films.

Methods: Chitosan and starch edible films were formulated with essential oil concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 2.00, or 4.00%. Mold radial growth was evaluated inoculating spores in 2 different ways. Edible films were placed over inoculated agar, Film/Inoculum mode (F/I), or the edible films were first placed in the agar and then films were inoculated, Inoculum/Film mode (I/F). The modified Gompertz model adequately described growth curves.

Results: There was significant difference ($P < 0.05$) in growth parameters between the 2 modes of inoculation. Starch edible films exhibited better antifungal effectiveness (MIC 0.25%) than chitosan films (MIC values of 0.50%). A significant ($P < 0.05$) change of Gompertz parameters was observed among essential oil concentrations, increasing the lag phase and decreasing radial growth rates as oil concentration increased.

Significance: Starch and chitosan edible films added with Mexican oregano essential oil could inhibit the development on anthracnose in fruits surfaces.

P2-42 Sensitivity of *Mycobacterium bovis* to Common Beef Processing Interventions

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Introduction: Cattle infected with *Mycobacterium bovis*, the causative agent of bovine tuberculosis and a relevant zoonosis to humans, may be sent to slaughter before diagnosis of infection because of slow multiplication of the pathogen.

Purpose: This study evaluates multiple processing interventions to determine a best practice as a means to control the spread of *M. bovis* in the beef processing environment.

Methods: Four strains of *M. bovis* isolated from cattle were evaluated in vitro for their ability to survive when exposed to seven commonly used interventions for 30 s in suspension. Treatments consisted of hot water (HW) at 65°, 70°, 75°, 80° and 85°C; lactic acid (LA) at 2 and 5% used at 25° and 50°C; and at ambient temperature 500 and 1,200 ppm acidified sodium chlorite (ASC); 50 ppm chlorine (Cl) and commercial products containing either 220 ppm hydrogen peroxide and peroxyacetic acid (POAA), 300 ppm bromine (Br), or a blend of 2% citric, phosphoric and hydrochloric acids (FF). All effects were measured by most probable number (MPN) determinations and colony forming unit (CFU) counts then compared to the level of *M. bovis* remaining after control (25°C) treatments in saline.

Results: Treatments with Cl, FF and either concentration of LA at ambient temperatures did not reduce the number of *M. bovis*. However, treatment with either concentration of LA at 50°C, POAA, or Br reduced *M. bovis* by 1.5 to 2 log CFU when compared to the control ($P < 0.05$). Additionally, both concentrations of ASC and hot-water at ≥ 75°C reduced *M. bovis* by at least 3 log CFU ($P < 0.05$) when compared to controls. In a timed exposure to 75°C HW the reduction was observed at 5 sec.

Significance: The most effective methods to reduce *M. bovis* viability were treatment with HW at or above 75°C and ASC at 500 or more ppm. Additional studies are recommended to evaluate efficacy of these treatments on carcass surfaces.

P2-43 Bacteriocinogenic *Lactococcus lactis* subsp. lactis Isolated from Goat Milk – Evidence for Expression of Nisin Z and Evaluation of the Probiotic Potential

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Introduction: The exploration of naturally occurring antimicrobials in food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern about microbial resistance toward conventional preservatives. Lactic acid bacteria capable of producing bacteriocins have technological applications in dairy products biopreservation. Bacteriocinogenic strains that present probiotic potential have an additional interest as they confer health benefits to the consumer.

Purpose: The purpose of this study was to characterize the bacteriocin produced by the strain *Lactococcus lactis* subsp. *lactis* DF04Mi, isolated from goat milk, investigate its potential probiotic properties and determine the genetic factors for bacteriocin production.

Methods: The production of bacteriocin was studied at 30°C and 37°C. Resistance of the bacteriocin to enzymes, detergents, various pHs and temperatures was evaluated. Its effect on several strains of *Listeria monocytogenes* and other foodborne pathogens was determined. The partial bacteriocin operon was sequenced and expression investigated. Growth of strain DF04Mi in different pH values, presence of ox-bile and medicines used for human and animal therapy was explored.

Results: Strain DF4Mi produced bacteriocin sensitive to proteolytic enzymes, resistant to heat and pH extremes and not affected by SDS, Tween 20, Tween 80, EDTA or NaCl. In the search for the nisin gene, the sequenced PCR product presented 100% homology to nisin Z. The bacteriocin was active against a large variety of foodborne pathogens and lactic acid bacteria, on a genus-dependent format. The strain presented potential probiotic properties, as it was resistant to acidic pH and ox-bile, presented low level of co-aggregation with *L. monocytogenes* and was highly sensitive to most antibiotics and affected by a limited number of drugs from different generic groups.

Significance: L. lactis DF04Mi strain, isolated from goat milk, produces antilisterial bacteriocins and presents potential probiotic properties, thus presents interesting potential for technological applications in the dairy industry. Acknowledgments: CNPq, CAPES, FAPESP

P2-44 Molecular Characterization of Antibiotic Resistance and Virulence Genotype of *Enterococcus faecalis* Isolates from Different Retail Meats

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Introduction: Enterococcus faecalis can be found in animal and human intestinal tracts, soil, plants, and water. It is also an important leading cause of nosocomial infections. The use of antimicrobial agents in livestock and poultry production can promote antibiotic resistance; however, little is known about the molecular ecology and genotype distribution of enterococci isolates from retail meats.

Purpose: The objective of this study was to analyze the genotype of *E. faecalis* isolated from retail meats from different stores in Alberta.

Methods: A total of 466 *E. faecalis* strains isolated from retail meat samples (chicken = 192, beef = 95, pork = 92 and turkey = 81) were identified by API 20 Strep and PCR. Susceptibility to antibiotics was performed using the Sensititre test with the NARMS's plate for Gram-positive bacteria using CLSI's breakpoints. A microarray carrying 70 taxonomic, 18 virulence and 177 antibiotic resistance gene probes was constructed and used to determine their genotype.

Results: None of the isolates were resistant to ciprofloxacin, daptomycin, linezolid and vancomycin. Varying degrees of resistance to other tested antibiotics was noted with resistance to lincomycin (97%) and quinupristin/dalfopristin (95%) being the most prevalent. Tetracycline, macrolide and aminoglycoside resistance was more frequent within poultry isolates with several strains displaying resistance to multiple antibiotics. The most common multiple resistance phenotype was erythromycin-tylosin-lincomycin-kanamycin-streptomycin-gentamycin-tetracycline-quinupristin/dalfopristin. Fourteen isolates were resistant to tigecycline recently recommended for approval in the USA. The identified resistance genes include aminoglycoside (*aac, aacA/aphD, aphA, aadE, aphA3*), macrolide (*ermB, ermAM*), tetracycline (*tetM, tetL, tetO*), streptogramin (*sat*), bacitracin (*bcrR*) and lincosamide (*linB*). Important virulence genes (*ace, agg, agrBfs, cad1, cAM373, cCF10, cob, cpd1, CylABLM, efaAfs, enlA and gelE*) were found especially in poultry isolates indicating their pathogenic potential. The enterococcal surface protein (*esp*) gene was detected in 5, 3 and 1 pork, beef and chicken isolates, respectively. One pork isolate was multi-resistant to 9 antibiotics and harbored 10 and 14 resistance and virulence genes, respectively.

Significance: Given our current knowledge related to the virulence potential and gene dissemination capacity of this bacterium, the high prevalence of virulence and antibiotics resistance genes of human importance in our retail meat *E. faecalis* isolates are of concern to public health.

P2-45 Antiviral and Protective Mechanisms of Korean Red Ginseng for Norovirus Surrogates

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Introduction: Korean red ginseng (KRG) is a well-known oriental herbal medicine for various beneficial effects. The antiviral effect of KRG is reported in several previous studies. However, antiviral activities of KRG against foodborne viruses has not been mentioned so far.

Purpose: The aim of this study was to investigate the antiviral effect and protective mechanism of KRG against norovirus surrogates, feline calicivirus (FCV) and murine norovirus (MNV).

Methods: To investigate the antiviral effect of KRG and ginsenosides, various concentration (0-10 μg/mL) of KRG and ginsenoside Rb1 and Rg1 were treated in CRFK (Crandell-Reese Feline Kidney) and RAW 264.7 cells and then, inoculated FCV, MNV respectively. To demonstrate these mechanism, 10 μg/mL total saponin of KRG, or ginsenosides, RNA and protein were extracted from CRFK and RAW 264.7, respectively. Expression of antiviral cytokines (interferon-alpha, and Mx) and tumor necrosis factor (TNF)-alpha was measured using reverse transcription-polymerase chain reaction and western blot assay.

Results: FCV and MNV significantly decreased in CRFK and RAW 264.7 treated with KRG, ginsenoside Rb1 and Rg1. In CRFK, expression of IFN- and Mx mRNA were increased from 8 hours to 48 hours after treatment of KRG and purified ginsenosides. Expression of Mx protein was also identified by western blot at 48 hours after treatment of KRG and ginsenosides. In RAW 264.7 cell, IFN- mRNA was detected from 12 hours to 48 hours after treated KRG and ginsenosides. Expression of TNF-alpha was not detected in all groups.

Significance: Expression of antiviral cytokines on the cells treated with KRG and purified ginsenosides contributes to antiviral mechanism. Antiviral activity of KRG against human enteric viruses should be investigated in further research.

P2-46 Reduction of Norovirus Surrogates during the Fermentation of Dongchimi

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Introduction: Dongchimi is a type of Kimchi which is a Korean traditional fermented food. Its fermentation is mediated by lactic acid bacteria. Norovirus is the most frequent foodborne disease worldwide in winter. However, the survival of foodborne viruses during the fermentation of Dongchimi has not been examined.

Purpose: This study aimed to examine the survival of FCV and MNV norovirus surrogates during the fermentation process of Dongchimi.

Methods: Feline calicivirus (FCV) and murine norovirus (MNV) were cultured and prepared. Dongchimi samples prepared by the traditional recipe were spiked with FCV or MNV, respectively. Fermentation was performed at 10°C for 20 days. Titration of FCV and MNV were measured at 1, 2, 3, 5, 7, 10, 15, 20 post-fermentation days (PFDs). The number of lactic acid bacteria, pH, and acidity were also checked.

Results: Titration of FCV was significantly decreased at 7 PFDs and not detected from Dongchimi samples in 15 and 20 PFDs. With MNV experiment, a 1.57 log reduction of MNV was observed at 20 PFDs. Lactic acid bacteria reached 7.70 log CFU/mL at 2 PFDs and pH significantly dropped at 5 FPDs.

Significance: Fermentation by lactic acid bacteria caused the significant reduction of norovirus surrogates in Dongchimi. Antiviral mechanism should be performed in further studies.

P2-47 Production and Characterization of Quail Egg Yolk Immunoglobulin Y (IgY) against *Vibrio parahaemolyticus* and *Vibrio vulnificus*

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Introduction: Vibrios are marine bacteria occurring naturally in the estuarine environment. They are foodborne pathogens causing food poisoning resulting in gastroenteritis in human.

Purpose: This study produced quail egg yolk immunoglobulin Y (IgY) against *Vibrio parahaemolyticus* and *Vibrio vulnificus* and characterized the produced antibodies using Enzyme Linked Immunosorbent Assay (ELISA) and Western blot.

Methods: To obtain IgY against *V. parahaemolyticus* and *V. vulnificus*, formalin-killed (FKVP, FKVV) and heat-killed (HKVP, HKVV) *Vibrio* immunogens (1.0 x 10^9 CFU/ml) along with Freund's adjuvant were intra-muscularly immunized six times into quail thigh muscles at two week intervals. Eggs were collected daily and IgY was purified weekly by water dilution-ammonium sulphate precipitation method. After adjusting the concentration to 1mg/ml by protein assay, IgY activity was determined by Indirect ELISA. The IgY with higher activity was selected for optimization of ELISA and cross reactivity studies.

Results: Of the four kinds of immunogens (FKVP, FKVV, HKVP, HKVV), formalin-killed immunogens (FKVP, FKVV) induced high humoral immune response for both *V. parahaemolyticus* and *V. vulnificus* over heat-killed immunogens (HKVP, HKVV). High IgY activity was obtained at the 9th week for all immunogens immunized. Of the four kinds of antibodies tested, HKVP IgY and FKVV IgY showed high sensitivity and specificity to *V. parahaemolyticus* and *V. vulnificus*, respectively, and hence, were selected for the development and optimization of indirect ELISA. Detection limits of the indirect ELISA using the produced IgYs were 10^5 CFU/ml for *V. parahaemolyticus* and 10^6 CFU/ml for *V. vulnificus*. The developed antibodies showed high binding affinity to their corresponding antigens, very little cross reactivity to *Staphylococcus aureus* and not other bacteria strains, a phenomenon which was also observed in Western blot.

Significance: The produced antibodies can be used for passive immunization to prevent infections caused by *V. parahaemolyticus* or *V. vulnificus* and also for the detection of these pathogens in food samples.

P2-48 Influence of Encapsulation on Bacteriocin Production by *Lactobacillus curvatus* MBSa2 in Broth Presenting Different Values of Water Activity (a_w), pH and Temperature

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Introduction: The success of the use of bacteriocinogenic lactic acid bacteria (LAB) as food biopreservatives depends on their survival and capability of producing bacteriocin in the food. Encapsulation of LAB in calcium-alginate beads is one alternative for maintaining high viable cell concentration. It is known that bacteriocin production in a food is influenced by several intrinsic and extrinsic factors. However, little is known about the degree of protection provided by the encapsulation process against the effects of these factors on bacteriocin production by LAB.

Purpose: This study aimed to evaluate the influence of encapsulation of *Lactobacillus curvatus* MBSa2 on bacteriocin production in MRS broth presenting values of water activity (a_w), pH and temperature that simulates those encountered during maturation of salami.

Methods: The study was carried out with *Lactobacillus curvatus* MBSa2, a bacteriocin-producing LAB isolated from salami. The strain was cultured in MRS broth at 30°C by 24 h, harvested by centrifugation and washed with peptone water (0.1%). The cells were encapsulated by adding 2% alginate and dripping the mixture in 100 mM $CaCl_2$ solution. Free and encapsulated cells were added separately to MRS broth with a_w adjusted to 0.97, 0.90 or 0.85 adding proper amounts of NaCl, and incubated at 30°C. They were also added to MRS with pH adjusted to 6.0, 5.5, 5.0 or 4.5, and incubated at 30°C, and to plain MRS incubated at 30°, 24° and 18°C. Counts of viable *L. curvatus* and levels of bacteriocin production were determined after 1, 3, 7 and 14 days.

Results: Maximal bacteriocin production (1862AU/log CFU) was obtained in MRS broth at pH 6 incubated at 30°C for 14 days. For all tested pH, free cells produced more bacteriocin than encapsulated cells. Production of bacteriocin by encapsulated cells was higher than by free cells when the a_w was 0.97. No bacteriocin production was detected when a_w was 0.90 or 0.85, by free or encapsulated cells. Bacteriocin production at 30°C was higher than at 24°C or 18°C.

Significance: Results indicate that encapsulated *L. curvatus* MBSa2 is able to produce bacteriocin in conditions that simulate maturation of salami. Production of bacteriocin during maturation is an additional hurdle for ensuring the safety of this product. Acknowledgments: FAPESP, CAPES and CNPq.

P2-49 Decontamination of Green Onions and Spinach Using Gaseous Ethyl Pyruvate

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Introduction: Foodborne illnesses associated with fresh produce continue to be a major concern as the consumer's demand for healthier and non-thermally processed food increases. However, many commercial disinfectants and antimicrobials are not effective against pathogens that may be internalized in the produce tissue.

Purpose: The objective of this study was to evaluate gaseous ethyl pyruvate (CAS 617-35-6) as a safe, alternative antimicrobial agent for the decontamination of *Escherichia coli* O157:H7 on green onions and spinach.

Methods: Baby spinach leaves and green onions were inoculated with a five-strain cocktail of *E. coli* O157:H7(GFP) using the dip-inoculation method. Samples were treated with 0, 40, 100, and 400 µl/1000 cm^3 concentration of gaseous ethyl pyruvate in an enclosed container. The efficacy of ethyl pyruvate on *E. coli* O157:H7(GFP) was monitored over the course of 7 days at 4°C and 10°C.

Results: The lowest concentration of ethyl pyruvate (40 µl/1000 cm^3) resulted in a 1.5 ± 0.5 log CFU/g reduction of *E. coli* O157:H7 (GFP) on green onions at 4°C in 7 days, and 2.5 ± 0.5 log CFU/g at 10°C in 5 days ($P < 0.05$). In baby spinach, the same concentration and incubation time resulted in 0.7 ± 0.3 log CFU/g and 1.5 ± 0.3 log CFU/g ($P < 0.05$) of *E. coli* O157:H7 (GFP) at 4°C in 7 days and 10°C in 5 days, respectively. On green onions, the highest concentration of ethyl pyruvate (400 µl/1000 cm^3) significantly reduced the population of *E. coli* O157:H7 by 5.0 ± 0.2 log in 7 days at 4°C, and 3 days at 10°C ($P < 0.05$). It was also determined that the same concentration was significantly effective in reducing *E. coli* O157:H7(GFP) populations by 4.4 ± 0.3 log at 4°C in 7 days and > 6 log at 10°C in 3 days with baby spinach ($P < 0.05$).

Significance: These results indicate that ethyl pyruvate is an effective antimicrobial that could be used to enhance the safety of fresh produce.

P2-50 Effect of Combined Process TiO2-UV Photo-catalytic Reaction and High Hydrostatic Pressure on Quality of Angelica Juice

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Introduction: Maintenance of quality and microbial control are problems of the unpasteurized fresh vegetable juice industry. Angelica (*Angelica keiskei*) juice is a popular health drink in Japan and Korea due to its nutritive quality, and is usually consumed as a fresh juice within 2 days. High hydrostatic pressure (HHP) is an alternative to heat for food preservation. Effects of TiO2-UV photocatalytic reaction (TPR) on in-water disinfection of fresh-cut vegetables were recently investigated, and its disinfection effect was significantly higher than other non-thermal disinfection methods. The TPR and HHP can be combined for a synergistic effect.

Purpose: The TPR was evaluated as a sequentially combined process with HHP in processing angelica juice for microorganisms, enzymes, and other physical properties.

Methods: Fresh angelica (*Angelica keiskei*) was cut into pieces and rinsed with running tap water. The angelica was divided into two groups. One group was treated with a TiO2-UVC photocatalytic reactor for 20 min, while another group was untreated. Angelica juice was obtained with an automatic juice extractor; each 50 ml was packed in a PE pouch and divided into two groups. One group was pressurized at 550 MPa for 1 min (two times) using a HHP equipment, while another group was untreated. Microorganisms and enzymes of the four groups (control, TPR, HHP, and TPR-HHP) were examined, and changes in the quality parameters were observed over an 8-day storage period at 4°C.

Results: Coliform bacteria, *Pseudomonas*, and yeast & mold were completely inactivated by HHP or TPR-HHP treatment. TPR-HHP treatment showed significantly higher reduction in counts of *Bacillus cereus* than HHP treatment only. Total aerobic bacteria counts were reduced by 1.3, 4.4, and 5.1 log CFU/ml with TPR, HHP, and TPR-HHP treatments, respectively. Total counts of control and TPR-treated juice increased to above 6 log CFU/ml within 2-day storage, while HHP and TPR-HHP treated juice maintained below 4 log CFU/ml even after 8-day storage. Quality-related enzyme activity decreased significantly by the HHP treatment.

Significance: This study indicates that pasteurization of fresh angelica juice by a sequentially combined treatment of TPR and HHP was possible.

P2-51 Disinfection Effects of the Continuous TiO2-UV Reactor on Microorganisms in Liquid Foods

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Introduction: Liquid foods such as fruit juice can be decomposed by microbial growth. TiO2-UV photocatalytic reaction has been used for removing organic materials or pollutants present in water or waste-water. Recently the TiO2-UV photocatalytic reaction has been investigated as a non-thermal disinfection method for foods. Novel continuous TiO2-UV photocatalytic reaction systems can be developed for the disinfection of liquid foods.

Purpose: Disinfection effects of a continuous TiO2-UVC photocatalytic reactor on microorganisms in liquid foods (water, clarified apple juice, and standard vinegar) were investigated.

Methods: The continuous photocatalytic TiO2-UVC reactor unit consisted of a stainless-steel chamber and a UVC lamp surrounded by a TiO2-coated quartz tube (or a quartz tube without coating for UVC only). Four units were connected to each other to allow a continuous flow. A liquid

food was circulated through the space between inner parts of chambers and quartz tubes at a flow rate of 80, 170, 360 and 750 ml/min to obtain surface area of 560, 1,120, 1,680 and 2,240 cm^2. *E. coli* O157:H7, *Saccharomyces cerevisiae*, *Listeria innocua*, *Acetobacter* spp. (*A. aceti*, *A. pasteurianus*, and *A. xylinum*) were inoculated into the liquid foods and viable cells were enumerated after each disinfection process.

Results: E. coli O157:H7, *A. aceti*, *A. pasteurianus*, and *A. xylinum* cells (initially 6-7 log CFU/ml) inoculated in apple juice or distilled water were completely inactivated by the continuous TiO2-UVC photocatalytic reactor within surface area of 2,240 cm^2. Reduction of 2 to 6 log CFU/ml was obtained for *S. cerevisiae* and *L. innocua* within 8 to 30 min (corresponding to flow rate of 80 to 750 ml/min). Disinfection effects of the TiO2-UVC photocatalytic reaction were significantly higher than that of UVC only.

Significance: This study suggested that the continuous TiO2/UVC photocatalytic reactor is applicable to non-thermal pasteurization of liquid foods.

P2-52 Organic Pecan Shells as a Source for Antimicrobials

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Introduction: The threat caused by *Listeria monocytogenes* and *Salmonella* Typhimurium is recognized as an important food safety issue in the U.S., especially for the more susceptible members of our population – children, the elderly and those with weakened immune systems. Each year in the U.S., listeriosis causes an estimated 2,500 persons to become seriously ill, and 500 of these illness lead to death. *Salmonella* has been the leading cause of human gastroenteritis. Salmonellosis causes an estimated 1.4 million cases of foodborne illness and more than 500 deaths annually in the United States. Despite these potential threats, consumers are growing even more concerned about the use of harsh chemical antimicrobials in their food. This creates a difficult situation for manufactures wishing both to ensure a safe food supply, and also wanting to satisfy consumer demands for unadulterated food. These demands have lead to the search for alternative antimicrobials.

Purpose: The purpose of our current research was to develop an effective, natural antimicrobial derived from an all-natural by-product, such as organic pecan shells.

Methods: Pecan shells were extracted using a range of solvent polarities (water, acetic acid, methanol and hexane) and antimicrobial activity of these extracts were evaluated. The different extracts were tested for their effectiveness on inhibiting growth of *L. monocytogenes* and *S.* Typhimurium using a serial dilution (48% to 0.375%) in a 96-well plate.

Results: The minimum inhibitory concentrations (MIC) of the extracts to inhibit growth of *L. monocytogenes* and *S.* Typhimurium ranged from 0.75% to 48%. For the extracts obtained by water and hexane as solvents, MICs were between 12 and 48% for both *Listeria* and *Salmonella*. For methanol, MICs are low for *Listeria* (0.75%) but higher for *Salmonella* (24%). With acetic acid, low MIC values were obtained for both organisms (1.5% and 0.75%). The results further indicate that all 4 solvents are effective in extracting antimicrobials from pecan shells.

Significance: With manufacturers now aware of their consumers' growing aversions to conventional antimicrobials, these extracts from pecan shells may offer an effective alternative.

P2-53 Antimicrobial Activity of Pomegranate *(Punica granatum)* Extract against Foodborne Bacterial Pathogens

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Introduction: Certain natural products, including pomegranate, contain elevated levels of antioxidants which may also possess antimicrobial activity. Pomegranate, a fruit native to the traditional regions of Persia and the Middle East, is increasingly available in western countries and is a rich source of antioxidants.

Purpose: The purpose of the study was to determine the ability of aqueous extracts of pomegranate to inhibit growth of several gram-negative and gram-positive bacterial pathogens and determine any synergistic effect between extracts of pomegranate and other natural products known to possess elevated levels of antioxidants.

Methods: Juice from the fruits of pomegranates, cranberry, and barberry was obtained by high pressure extraction. Inhibitory activity was monitored by disc diffusion assay. HPLC analysis used a Zorbax B-C18 column, 250 x 4.6 mm. Potential synergistic antimicrobial activity of samples containing pomegranate and aqueous extracts of either cranberry, barberry, or oregano was determined at equivalent phenolic concentrations of 1mg/ml and compared to pomegranate alone.

Results: Several strains of each of five food-associated bacterial pathogens were readily inhibited by pomegranate extracts with average zones of inhibition of 0.80 cm (*Clostridium perfringens*), 0.90 cm (*Bacillus cereus*), 0.77 cm (*Staphylococcus aureus*), 0.70 cm (*L. monocytogenes*), and 0.84 cm (*Helicobacter pylori*) while *Escherichia coli* O157:H7 and *Salmonella enterica* were fully resistant. No significant difference ($P = 0.1$) in synergistic activity between pomegranate combined with each of the other natural products versus pomegranate alone was demonstrated. HPLC analysis profiles of pomegranate extracts demonstrated the presence of ellagic acid and other components consistent with polyphenolic compounds.

Significance: The results show that extracts of whole pomegranate (juice) possess compounds which are clearly effective in inhibiting certain foodborne bacterial pathogens. The effectiveness against *H. pylori*, a gastric bacterial pathogen, suggests a potential novel approach in controlling this organism.

P2-54 Antibacterial Activity of Ethanol and Water Extract of Different Parts from *Sasa borealis*

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Introduction: Sasa borealis is part of the bamboo tree. *Sasa borealis* grows wild and many Koreans drink it as tea. Many studies have demonstrated that *Sasa borealis* has antioxidant activities. However, little work has been carried out on the antibacterial activities of *Sasa borealis*.

Purpose: This study investigated the antibacterial activities of ethanol and water extract toward the different parts (total, leaves, canes, roots) of *Sasa borealis* against *Staphylococcus aureus* and *Salmonella choleraesuis*.

Methods: Using survival curves, the kinetics of bacterial inactivation on extract exposure was followed 24h and in this way the MIC50 (minimum inhibitory concentration) values determined by broth micro-dilution assay were confirmed as the concentrations of extracts that inhibited bacterial growth.

Results: Sasa borealis extracts showed antibacterial activities against *Staphylococcus aureus* and *Salmonella choleraesuis*. Seventy % ethanol extract of *Sasa borealis* has stronger activities than 50% ethanol and water extract. In particular, all extracts of *Sasa borealis* have stronger antibacterial activities against *Salmonella choleraesuis* than *Staphylococcus aureus*. MIC50 of 70% ethanol extract of all parts of *Sasa borealis* were determined 100 ppm against *Staphylococcus aureus* and of all tested extracts of *Sasa borealis* were determined 100 ppm against *Salmonella choleraesuis*.

Significance: These results suggest extracts and fractions of *Sasa borealis* effectively inhibit bacterial growth and are useful as natural antibacterial agents.

P2-55 Evaluation of Biological Effects of Synthetic Thioflavanones as Novel Antimicrobial Agents

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Introduction: Various flavonoids have antimicrobial activity. Many studies have demonstrated that various classes of flavonoids have a broad range of biological activities. In contrast, little work has been carried out on sulfur containing flavones. Thioflavones have been reported to show potent antimicrobial and antimalarial activities.

Purpose: In this study, the antimicrobial activities of nine synthetic thioflavanone derivatives were investigated against eight bacteria and *Rizopus* spp.

Methods: Using broth microdilution assay and agar dilution assay, we have displayed survival curves. The kinetics of bacterial inactivation on exposure to synthetic thioflavanone derivatives was followed for 24 h and, in this way, the MIC (minimum inhibitory concentrations) determined by broth microdilution assay. MBC (minimum bactericidal concentrations) were determined by agar dilution assay.

Results: MIC values for test bacteria which were sensitive to the synthetic thioflavanone derivatives were in the range of 31.25-500 μg/mL. MBCs (minimum bactericidal concentrations) were determined by agar dilution assay and MBC values were confirmed 250-500 μg/mL.

Significance: All synthetic thioflavanone derivatives displayed potent antimicrobial activity against fungi.

P2-56 Inhibition of Foodborne Pathogens and Spoilage Organism in Dairy Dessert Using ε-polylysine

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Introduction: Listeria monocytogenes, Bacillus cereus and *Lactobacillus plantarum* have been reported to be pertinent foodborne pathogens and spoilage microorganisms associated with dairy products. ε-polylysine, a cationic antimicrobial, has excellent antimicrobial properties. Specific application of ε -polylysine was evaluated for antimicrobial efficacy against different microorganisms in a dairy dessert.

Purpose: The objective was to evaluate the antimicrobial efficacy of ε-polylysine in a dairy dessert against *Listeria monocytogenes, Bacillus cereus* and *Lactobacillus plantarum*.

Methods: The PuraQ® Xtend FX25, (ε-polylysine concentration 25%), (0, 0.02, 0.05, and 0.10% concentrations) and 0.1% potassium sorbate treatments were evaluated. *L. monocytogenes, B. cereus* and *Lb. plantarum* strains were independently inoculated to have ca. 2–3 log CFU/mL counts in the dairy dessert (pH 6.21, a_w 0.988) to evaluate the growth at 4, 7 and 10°C, respectively. The plating for each microorganism was done independently for 60 days.

Results: The *L. monocytogenes* growth data indicates that the ε-polylysine treatment at the max. tested level, arrested the growth of *L. monocytogenes* < 1 log CFU/mL after incubation for ca. 30 days and did not reach 2 log CFU/mL during the 60 days of incubation period. The *L. monocytogenes* counts reached >7 log CFU/mL in the 0.1% k-sorbate treatment. The *B. cereus* growth data indicates that the ε-polylysine treatments influenced the growth of the microorganism and decreased it to below detection limit (1.78 log CFU/mL) throughout the incubation period. The *Lb. plantarum* growth data demonstrate that the ε-polylysine treatments continually decreased the microbial counts by more than 1 log CFU/mL compared to the initial inoculation levels over the incubation period. These results demonstrate strong antimicrobial efficacy of ε-polylysine in food applications.

Significance: This research substantiates the antimicrobial efficacy of ε-polylysine in the dairy dessert and provides the food industry with a highly effective antimicrobial to assure the food safety of the dairy products.

P2-57 The Isolation of Bacterial Growth Suppressing and Growth Promoting Compounds from Jalapeno Pepper Extract Using Liquid Chromatography

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Introduction: Studies suggest that chile peppers (*Capsicum* spp.) may possess antibacterial properties, although compound(s) responsible for this activity have yet to be identified. Capsaicin, the compound responsible for the sensation of heat associated with hot peppers, has been investigated for antibacterial activity with conflicting results.

Purpose: The purpose of this research was to separate and collect compounds from jalapeno pepper extract using high-performance liquid chromatography (HPLC) and determine which extract fractions affected the growth of selected foodborne bacterial pathogens.

Methods: Compounds from fresh jalapeno extract were collected as they eluted from a reverse-phase HPLC column, processed, and added to trypticase soy broth (TSB) for use in this study. Growth curves for *Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella enterica* grown in TSB with extract fractions added were generated over 72 h using a BioScreen-C growth curve machine. The effects of the jalapeno extracts on growth over time were determined using ANOVA and compared using Tukey's HSD.

Results: The addition of Fraction E (collected during 20–25 min of elution) to TSB resulted in significantly lower ($P < 0.05$) optical density (O.D.) readings than control growth curves for *L. monocytogenes* for up to 12 h, *S. enterica* for up to 24 h, and *E. coli* O157:H7 for up to 36 h. Fraction C (10–15 min elution) resulted in significantly higher O.D. readings than the control for *L. monocytogenes* throughout the duration of growth recorded (72 h). The only known standard that eluted off the column during the same timeframe as Fraction E was cinnamic acid, but further analysis determined that cinnamic acid is not the compound responsible for suppressed bacterial growth. Capsaicin was not recovered in the fractions that influenced growth of the bacteria observed.

Significance: This study contributes to the understanding of *Capsicum* spp. as a source of natural antibacterial compounds. Isolated compounds should be further investigated to understand their potential role in food safety applications in the future.

P2-58 Development of an Antimicrobial Alginate based Film: *in vitro* and *in situ* Tests

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Introduction: The use of biodegradable packaging such as edible films and coatings are an alternative to the use of non-recyclable packaging. The incorporation of antimicrobial substances in packages aims at reducing food microbial contamination among which essential oils (EO) have received special attention, being natural and attending consumer demand.

Purpose: To develop an alginate-based film with clove EO as an antimicrobial agent, evaluating the addition of different concentrations of calcium chloride ($CaCl_2$) in different steps of film production, and to assess the *in vitro* and *in situ* antimicrobial activity of films against *Listeria monocytogenes* and *Pseudomonas* spp isolated from chicken meat.

Methods: The experimental design adopted was the central composite rotational 2^2 with the independent variables $CaCl_2$ (0.02 to 0.1%) and clove EO (0.2 to 1.0%). Alginate-based films were prepared by mixing glycerin (2%) in distilled water prior to the EO emulsion addition. The

sodium alginate (3%) was then added under agitation and as a first crosslinking step the $CaCl_2$ was added and homogenized. After drying, the second crosslinking step with $CaCl_2$ was performed. The *in vitro* antimicrobial activity tests were carried out by the agar diffusion method using film disks placed on Mueller Hinton agar plates spiked with inoculum containing 10^5 to 10^6 UFC/mL of each bacterium tested. The *in situ* evaluation of the antimicrobial activity was performed with the formulation that showed the highest inhibition values *in vitro*. Samples of raw chicken meat were spiked with each bacterium (10^3 UFC/g) and then wrapped with the antimicrobial alginate film. The microbiological analyses were performed at the first and fifth days of storage at 7°C. Experimental data were analyzed using the software Statistica 7.0.

Results: For all the bacteria tested, the highest inhibition values were obtained with $CaCl_2$=0.0316% and clove EO=0.0884%. The film containing clove EO controlled *L. monocytogenes* in chicken meat compared to control samples ($P < 0.05$). However, for *Pseudomonas* spp an increase in population was observed for test and control ($P < 0.05$).

Significance: Besides being an alternative to replace non-recyclable packaging materials, the use of alginate-based films incorporated with clove EO plays an important role in antimicrobial packaging in controlling *L. monocytogenes* growth in refrigerated chicken products.

Acknowledgment: FAPESP.

P2-59 Disinfection of Fresh Produce with Atmospheric Plasma

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Introduction: There is an overwhelming need to develop technologies that will reduce spoilage of fresh produce and reduce the number of pathogens without altering taste or nutrition. Reduction of post-harvest losses of produce translates into a reduction in the cost of production, trade and distribution and provides consumers fresh and healthy fruits and vegetables.

Purpose: Atmospheric plasma technology creates transient, biocidal reactive chemical species to reduce the microbial population on fresh produce, minimizing foodborne illness outbreaks and increasing shelf life.

Methods: An atmospheric plasma system was developed to disinfect produce at different processing temperatures. Produce evaluated with plasma treatment was hand picked at a local hydroponics farm and included blueberries, strawberries, tomatoes, and kale.

Results: Produce inoculated with either *E. coli* O157:H7 (ATCC # 85150); *L. monocytogenes* (ATCC # 19115); a cocktail of *S.* Enteritidis and *S.* choleraesuis (ATCC # 10708) showed 3 to 4 log reductions in cell number when compared to untreated produce. Also, plasma treated strawberries that were inoculated with (10^8 CFU/ml) of pathogens showed 3.5 to 4 log reductions in cell number when compared to untreated controls, even after 5 days of storage at 4°C. Shelf-life studies showed immediately following plasma treatment, there was a sharp reduction in spoilage organisms, and on Day 7 of the test, the treated tomatoes stored at 4°C had 99% less flora on their surfaces than untreated tomatoes. Furthermore, there was little significant change in pH, color intensity, and texture in treated and control strawberries and tomatoes, even after 9 days of storage at 4°C. The plasma treated kale suffered severe drying.

Significance: Significantly improving the safety and freshness of produce will tremendously impact our society since fruits and vegetables are nutritionally necessary, and a cornerstone of a healthy diet.

P2-60 Survey of Food Safety Practices on Small to Medium-sized Farms and in Small Farmers' Markets

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Introduction: Some consumers view organic and locally grown foods as healthier, safer and better for the environment. However, little research has addressed food safety practices used on small to medium-sized farms or in small farmers' markets.

Purpose: The study surveyed farmers on small to medium-sized farms and managers of small farmers' markets in Georgia, Virginia and South Carolina to determine their current food safety practices.

Methods: Surveys were developed, pre-tested and revised before implementation with the target audiences. Revised surveys were implemented via mail and the web to maximize participation, with reminders sent to non-respondents. Frequencies and percentages were calculated for all response variables.

Results: Data were collected from 226 farmers and 45 market managers. More than 55% of farmers use manures including poultry, cattle, horse, goat, bat and humanure (composted human manure), and of these, >45% indicate using less than a 120-day waiting period between application and harvest. Over 25% use untested water sources for irrigation and 15% for washing produce. Approximately 50% harvest crops with bare hands. Over 40% do not sanitize surfaces that touch produce at the farm. Only 33% always clean transport containers between uses. Most farmers do have handwashing and/or bathroom facilities close to their fields and/or packing houses. Of market managers, over 40% have no food safety standards in place and only small percentages (≤11.1) ask farmers about specific conditions on the farm that could affect product safety. Less than 25% sanitize market surfaces, and only 11.1% always clean market containers between uses. Over 75% of markets offer no sanitation training to workers or vendors. However, 66% of the managers indicated they were interested in educational materials for their markets.

Significance: Unsafe practices identified on small to medium-sized farms and in small farmers' markets may put consumers at risk of foodborne illness and indicate a need for training.

P2-61 Evaluation of a Portable, Recycled Vertical Flow Constructed Wetland as a Low Cost Treatment System for Greywater Reuse in Food Production

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Introduction: Economically and environmentally sustainable wastewater treatment options are important tools in the reuse of greywater for food crop irrigation. Constructed wetlands (CWs) are an effective, low cost system for the remediation of bacterial contamination in greywater. However, current construction methods for CWs can incur large capital costs, prohibiting the implementation of this technology in water-stressed communities. Therefore, alternative methods of CW construction should be investigated.

Purpose: The purpose of this study was to develop and evaluate CWs with low surface area requirements, and low capital construction costs, which would achieve biologically acceptable contaminant removal efficiencies.

Methods: A total of four 1 m^2, portable, recycled vertical flow constructed wetlands (RVFCW) were built for this study. Two RVFCWs were built with recycled, polyethylene terephthalate (PET) plastic as the primary wetland bed media, and two more were constructed with traditional volcanic tuff. The wetlands were dosed with 350 l d^{-1} of greywater six times during a three month period. Water samples were taken at four different locations within the treatment stream, and analyzed for nine parameters including: total plate count (TPC), fecal coliforms (FC), and total organic carbon (TOC).

Results: The RVFCWs achieved 2 log reduction for TPC ($P < 0.0001$), and 3 log reduction for FC ($P < 0.0001$), while no significant differences were observed between the RVFCWs constructed with recycled PET and volcanic tuff ($P > 0.05$). In addition, the RVFCWs achieved 51.5% removal of TOC ($P < 0.0001$), with no statistical differences found between RVFCW types ($P > 0.05$).

Significance: The results of this study indicate that RVFCWs can achieve appreciable removal efficiencies for TPC, FC, and TOC. Therefore, RVFCWs may be a viable, low cost, minimal technology, polishing step for treating greywater to reuse as irrigation water. In addition, RVFCW construction cost can be drastically reduced by utilizing recycled PET plastic as a primary wetland bed media without compromising treatment efficacy.

P2-62 The Use of Zero-valent Iron and Biosand Filtration to Inactivate *Escherichia coli* O157:H7 in Irrigation Water

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Introduction: Foodborne pathogens can be disseminated to produce through contaminated irrigation water. Effective, low cost mitigation strategies, like biosand and zero-valent iron (ZVI) filtration, may be effective in decontaminating irrigation water.

Purpose: To determine the effectiveness of biosand and zero-valent iron filters in removing *E. coli* O157:H7 from water.

Methods: Commercial HydrAid biosand filters were built as recommended containing gravel and coarse sand. Columns were then modified to contain either fine sand only (S), a combination of ZVI and fine sand at a 1:1 ratio (SI), or ZVI only (ZVI). Columns were prepared by passing 20L of uninoculated ground water through each column every day for 2 weeks and flow rates were determined. *E. coli* O157:H7, cultured in a manure slurry, was added to the columns at 8.5 log CFU/100 ml in 20L of water. At specific time points, surviving *E. coli* O157:H7 in filtered water were determined by collection on 0.45µm filters, and enumeration on MacConkey agar. Following column contamination, uninoculated groundwater (20 L) was fed into each column for up to 30 days and analyzed for *E. coli* O157:H7.

Results: E. coli O157:H7 counts recovered from S columns from Day 0 through 7 were TNTC (>300 CFU/100 ml); however, *E. coli* O157:H7 counts from SI columns on Day 2, 7 and 14 declined to 3.97, 1.79 and 0.34 log CFU/100 ml, respectively. Counts in ZVI columns on Days 2, 7, and 14 declined to 3.44, 0.46, and 1 log CFU/100ml, respectively. By Days 8, 14, 21 and 28, counts recovered from S columns were 4.00, 5.98, 3.20, and 2.22 log CFU/100ml, respectively. No *E. coli* O157:H7 was recovered from ZVI by Day 14 or from SI by Day 17. *E. coli* O157:H7 were recovered from S columns through Day 31.

Significance: This preliminary works shows that ZVI columns were more efficient than biosand columns in removing *E. coli* O157:H7 from contaminated water.

P2-63 *Escherichia coli* Populations in Irrigation Water and Broccoli, Cauliflower and Celery Crops: Farm-scale Experiment

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Introduction: Irrigation water used for crop irrigation can be contaminated by indicator and pathogenic microorganisms. Little is known on the persistence of such microorganisms on crops at the farm scale.

Purpose: The purpose of this study was to determine *E. coli* prevalence on irrigated broccoli, celery and cauliflower with surface or groundwater under field conditions.

Methods: A survey was conducted in southern Quebec (Canada) in 2006 and 2007. Irrigation water samples were taken during every irrigation performed during the month preceding harvest in broccoli (n=34), cauliflower (n=9) and celery (n=25) fields. Ten vegetables were sampled at harvest time in each field and analyzed separately. *E. coli* populations in water were determined using the mTEC modified membrane filtration method whereas vegetable samples were processed using both direct plating on Petrifilms™ count plates (2006 and 2007) and enrichment with the Colilert® medium (2007).

Results: E. coli populations in irrigation water ranged from 0 to 1099 CFU/100 mL (geometric mean= 8 CFU/100 mL) (n=103). 86% of water samples were under 100 CFU/100 mL (geometric mean= 5 CFU/100 mL). The majority (92%) of water samples came from groundwater stored in ponds (geometric mean= 8 CFU/100 mL). No link could be established between the water source and *E. coli* prevalence on vegetables. Overall, *E. coli* populations could be quantified in 2 celery and 2 broccoli samples (n=679). Bacteria could be detected in 21 celery samples using the enrichment method, but they were not recovered in other crops (n=280). Celery morphology as well as contact with soil could explain these results. All *E. coli*-positive vegetables were grown in fields irrigated with water containing less than 100 CFU/100 mL during the month preceding harvest.

Significance: This study highlights the limited prevalence of *E. coli* on broccoli, cauliflower and celery crops after irrigation. Results have also shown that irrigation water source (surface or groundwater) had no impact on *E. coli* prevalence in water and vegetables under the conditions of this experiment.

P2-64 Absence of Direct Association between Coliforms and *Escherichia coli* in Irrigation Water and on Produce

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Introduction: Contaminated irrigation water is a potential route of pre-harvest pathogen contamination of vegetables. Hence, a number of organizations have recommended water quality guidelines. However, the relationship between irrigation water quality and microbial contamination of irrigated vegetables is not clearly defined.

Purpose: Determine the association between microbial quality indicators (coliforms and *Escherichia coli*) in irrigation water and on irrigated vegetables.

Methods: Correlation coefficients were used to determine associations between indicator organisms in irrigation water and on produce. Data analyzed included original results from a cross-sectional study conducted in Ohio, USA, during the Summer 2009 and information presented in two previously published studies identified through a systematic review process (Studies A: France and B: Portugal).

Results: In the cross-sectional study, there were no significant correlations between fecal indicators (total coliforms, *E. coli*) on leafy greens (lettuce and parsley, n=118), fruit (tomatoes and pepper, n=33) and irrigation water used ($P > 0.05$). Likewise, no significant positive associations in total coliform, fecal coliform or fecal streptococci were identified between lettuce and waste irrigation water (n=12; Study A), nor fecal coliforms on lettuce and irrigation water (n=16; Study B).

Significance: The concentration of indicator organisms on produce cannot be accurately predicted solely from a single measure of irrigation water quality. In the absence of additional information, such as the rate of pathogen population decline on specific plants under defined environmental conditions, method of water application, and the irrigation-to-harvest interval, the use of a single microbial water quality parameter as an indicator of produce safety is of limited value.

P2-65 Bacterial Pathogens in Irrigation Water and on Produce are Affected by Certain Predictor Variables

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Introduction: Contaminated irrigation water sources have been reported as a major way by which fruits and vegetables become contaminated with bacterial pathogens. For improved produce safety, it is essential that physico-chemical properties of water or other indicator organisms different from the common ones, i.e., fecal coliforms, fecal streptococci and *E. coli* are used used for monitoring the microbiological safety of water and produce.

Purpose: Our goal was to use logistic regression analysis and some predictor variables to predict the presence of selected bacterial pathogens, i.e., *Salmonella* spp., *L. monocytogenes* and intestinal *Enterococcus* in irrigation water and vegetables. This is because determination of the presence of all pathogens in irrigation water and vegetables could be costly and also time consuming

Methods: The associations of the occurrence of *L. monocytogenes*, *Salmonella* spp and intestinal *Enterococcus* in irrigation water and vegetables were explored using binary logistic regression analysis. For this analysis, we dichotomised the dependent variables, *L. monocytogenes*, *Salmonella* spp and intestinal *Enterococcus* where values for absence were coded as '0' while values for presence were coded as '1'. For prediction of the three bacterial pathogens in irrigation water, four predictor variables (coliforms, fecal coliforms, COD and turbidity) were taken into the model. On the other hand, ACC, *S. aureus*, location, ASF, AnSF, coliforms and fecal coliforms were used as predictor variables in the model for prediction of the bacterial pathogens in vegetables. The resulting regression coefficients quantified the type of association between the predictor variable and the respective dependent variable. A *P*-value of ≤ 0.05 was considered statistically significant and all reported *P*-values were two-tailed.

Results: It was evident that COD was statistically reliable to predict *L. monocytogenes*, turbidity, reliable to predict intestinal *Enterococcus* and fecal coliforms and coliforms, and reliable to predict *Salmonella* in irrigation water. Also, while the regression analysis showed that the aerobic colony count (ACC) and aerobic sporeformer count (AnSF) could be used to predict *Salmonella* and intestinal *Enteroccus* in vegetables, *S. aureus* and ACC were indicated to be significant parameters in predicting *L. monocytogenes* on vegetables.

Significance: This work showed that in addition to the common indicators, i.e., *E. coli*, fecal coliforms, and fecal *Streptococci*, the microbiological quality of irrigation water and vegetables might be indicated after physico-chemical properties and ACC.

P2-66 Antimicrobial Effects of Vanillin, Ethyl Vanillin and Vanillic Acid Against *Cronobacter* spp.

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Introduction: Cronobacter species cause severe illnesses in newborns and infants. Contaminated powdered infant formulas are a major source of infection. Inactivation during rehydration or in prepared formula are desirable strategies for the control of this emerging foodborne pathogen.

Purpose: The purpose of this research was to determine whether the common flavoring agents vanillin, ethyl vanillin and vanillic acid inhibit the growth and/or reduce the heat resistance of *Cronobacter* spp. in a model system.

Methods: A use dilution method performed in microtiter plates was employed to determine minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations against seven *Cronobacter* isolates (three *C. sakazakii* strains and one of *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C.* genomospecies 1) in ¼ strength tryptic soy broth adjusted to pH 5, 6 and 7 following supplementation with vanillin, ethyl vanillin or vanillic acid. The effect of supplementation with each compound at MBC concentration on the heat resistance (D value at 58 °C) of a cocktail containing three *C. sakazakii* strains was examined in ¼ strength TSB adjusted to pH 5 or 6.

Results: All three compounds exhibited pH- and temperature-dependent bacteriostatic and bactericidal effects against all *Cronobacter* spp. and the capacity to decrease the heat resistance of a *C. sakazakii* cocktail. D-values were reduced from 15.13 ± 0.54 min to 1.74 ± 0.10 min at pH 6 and 0.98 ± 0.02 min at pH 5 in medium containing 6 mg/ml vanillic acid, 0.84 ± .04 min at pH 6 and 0.93 ± 0.01 min at pH 5 with 4 mg/ml vanillin, and 0.50 ± 0.02 at pH 6 and 0.63 ± 0.01 min at pH 5 with 3 mg/ml ethyl vanillin. Overall, ethyl vanillin had the strongest antimicrobial activity and the greatest effect on the heat resistance of *Cronobacter*.

Significance: These findings suggest that vanillin, ethyl vanillin or vanillic acid could be useful additives for the control of *Cronobacter* spp. in rehydrated infant formula.

P2-67 *Escherichia coli* Population Dynamics on Romaine Lettuce Contaminated with Poultry Manure

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Introduction: Human pathogens undergo time-dependent decay when they are released into the agricultural environment or on leafy vegetable crops. Microbiological methods that yield estimates of time elapsed since a contamination event could therefore provide useful information about the risk of contamination with human pathogens.

Purpose: The purpose of this study was to determine if changes in *E. coli* population dynamics can provide an estimate of time elapsed since lettuce contamination with poultry manure.

Methods: Poultry manure slurry, *Salmonella* Typhimurium, *Listeria innocua* and *E. coli* O157:H7 were applied to the leaves of Romaine lettuce 5 weeks after emergence of plants grown in controlled environment chambers. *E. coli* populations were estimated on Violet Red Bile-MUG agar and selective plating procedures were used to detect the test bacteria for 5 weeks after inoculation. Changes in *E. coli* population diversity were examined by a PCR-based scheme that assigns individual isolates to one of four phylogenetic groups (A, B1, B2, and D).

Results: Salmonella Typhimurium, *Listeria innocua* and *E. coli* O157:H7 populations on lettuce leaves were reduced from 10^5 CFU/g to $<10^1$ CFU/g within 2 weeks after application of manure slurry but commensal *E. coli* populations decayed at much slower rates and $>10^1$ CFU/g remained after 5 weeks. *E. coli* from all four phylogenetic groups were recovered from manure slurry (10.2% A, 32.1% B1, 61.3% B2, 22.2% D). The relative proportion of these groups changed over time and 100% of isolates recovered from lettuce leaves after 5 weeks were from group B1.

Significance: This research suggests that *E. coli* from individual phylogenetic groups differ in their ability to survive on the lettuce surface. Phylogenetic characterization of *E. coli* could therefore provide a measure of time elapsed since contamination with a source of enteric microorganisms and improved assessments of the risk of contamination with human pathogens.

P2-68 Impact of Spinach Plant Maturity on Survival of *Escherichia coli* O157:H7 in Soil and Internal Transmission to the Plant

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Introduction: The presence of pathogens in the soil during plant growth is a potential source of contamination. While there are numerous studies indicating all potential sources of contamination pre- and post-harvest, there is little information about the effect of different stages of plant growth on survival of *E. coli* O157:H7 in the soil and its potential transmission to the plant.

Purpose: Determine if there is any potential effect of spinach plant growth and maturity on survival of *E. coli* O157:H7 in the soil and internal transmission to the plant.

Methods: Spinach seeds were sown in individual containers (Day 0). Treatments were: Control - uninoculated soil; IS0 - soil inoculated before planting; and IS7, IS14, and IS28 - soil inoculated 7, 14 and 28 days, respectively, after planting. Inoculated (n=5 plants/day) and control (n=3 plants/day) plants were collected every 7 days for 6 weeks starting from Day 0. Measurements included plant weight (not including roots) and enumeration and detection of *Escherichia coli* O157:H7 in the soil and plant shoots.

Results: Results indicated that although *Escherichia coli* O157:H7 survived longer in the soil when inoculated before planting, it was not internally transmitted to the shoot of the plant during growth. Internal transmission of *E. coli* O15:H7 from soil to shoot only occurred when the 28-day-old plants' soil was inoculated and that mostly occurred right after inoculation.

Significance: These findings indicated that older plants were more susceptible to internal contamination than younger plants when first exposed to this pathogen via soil inoculation.

P2-69 Pre-harvest Field Exposure of Seeds or Cut-back Baby Leafy Greens to *Escherichia coli* O157:H7: Potential for Internalization of Pathogen

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Introduction: Internalization of foodborne pathogens into sprouting seeds has been demonstrated in hydroponic or autoclaved soil systems. Cut surfaces of leafy greens have also been demonstrated under post-harvest conditions to serve as a site where pathogens may be internalized.

Purpose: To assess field-grown leafy greens for internalized *Escherichia coli* O157:H7 (O157) using two pre-harvest scenarios: 1) leafy green seeds germinated in contaminated soil; and 2) cut-back baby greens (to be grown for a second crop) sprayed with contaminated water.

Methods: In Scenario 1, compost was inoculated with GFP-labeled avirulent O157 and incorporated into soil of field plots (1 to 5 log CFU/g). Seeds of baby lettuce and baby spinach were sown in these plots and 13 to 39 days after sowing, seedlings (entire plant) were assayed for internalized populations of O157. In Scenario 2, baby lettuce and spinach were grown in field plots and harvested at maturity. Water contaminated with a low or high dose of GFP-labeled avirulent O157 (4.5 or 7 log CFU/ml, respectively) was sprayed on the cut-back plants either immediately after cutting or 1 or 5 days later. Assay for internalized O157 in aerial plant tissue occurred on 0, 2, and 14 days after spraying plants. A silver nitrate wash and sterile water rinse of plant tissue prior to grinding and analysis targeted internalized pathogen.

Results: In the seed study, lettuce and spinach seedlings did not harbor any internalized O157 at any sampling time. In the cut-back study, internalized O157 populations (4.0 log CFU/g) were similar on Day 0 in both spinach and lettuce plants sprayed with the high dose treatment. A delay between cutting and spraying did not impede initial internalization but did lead to accelerated decline in internalized populations for tissues sampled 2 days later. Internalized O157 could not be detected by enrichment in plant tissues sampled 2 days (low dose) and 14 days (high dose) after spraying.

Significance: Understanding the conditions under which pathogens could be internalized into plant tissues is essential for the development of management practices that would reduce the risk of pre-harvest contamination.

P2-70 *Salmonella* spp. and *Escherichia coli* O157 Presence and Indicator Organism Levels in an Open-surface Water Stream Used for Irrigation in Western Washington

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Introduction: Understanding relationships between pathogen presence and indicator organism levels in irrigation water is a critical agricultural issue.

Purpose: To examine a potential source of contamination, surface water used for irrigation in western Washington was monitored for pathogen presence and indicator organism levels over two years.

Methods: Water samples were collected 18 times throughout 2008 and 2009 (9 dates/yr) at 8 sites: near an irrigation pump (Site 7), at six upstream sites (Sites 1 to 6) and one slightly downstream (Site 8). Samples were quantified for fecal coliforms and generic *Escherichia coli* (*E. coli*) using a five-tube most probable number technique, and analyzed for pathogen presence (*E. coli* O157, *Salmonella* spp.). Washington Department of Ecology (WA-DOE) utilizes a water quality standard for secondary contact recreation water of < 2.3 log colonies/100 ml fecal coliforms and the Leafy Greens Marketing Agreement (LGMA) recommends <2.37 log MPN/100 ml generic *E. coli* for foliar irrigation.

Results: Sampling date within year and sampling site were significant factors for fecal coliforms ($P < 0.01$) and generic *E. coli* ($P < 0.01$ and $P < 0.05$, respectively). In 2008, fecal coliform levels were >2.5 log MPN/100 ml at 3 sampling periods (July, mid-September and November) and generic *E. coli* levels were <1.0 log MPN/100 ml at 3 sampling periods (early and mid-September and late October). In 2009, fecal coliform levels were >2.5 log MPN/100 ml at 4 sampling periods (June through September) and generic *E. coli* levels were <1 at 4 sampling periods (January, February, May, June). Sites 5, 6 and 7 (irrigation pump) were significantly higher in fecal coliforms (>2.6 log MPN/100 ml) and generic *E. coli* (>1.4 log MPN/100 ml) compared to Site 1, the farthest upstream (1.3 log MPN/100 ml fecal coliforms and 0.59 log MPN/100 ml generic *E. coli*). Almost half of the samples (46%, n=134) met both the WA-DOE fecal coliform standard and the LGMA generic *E. coli* standard. Alternatively, 43% exceeded only the WA-DOE standard and 10% exceeded both the WA-DOE and LGMA standards. Pathogens were detected in 7.5% of the samples (seven *E. coli* O157 and three *Salmonella*-positive samples). Six pathogen-positive samples were observed when both standards were met. One pathogen-positive sample occurred when both the WA-DOE and LGMA standards were exceeded, and three occurred when only the WA-DOE standard was exceeded.

Significance: Pathogens were detected in irrigation water samples that met the WA-DOE and LGMA water quality standards emphasizing that indicator organisms did not always predict pathogen risk.

P2-71 Comparison of Pathogen Contamination Routes of Spinach Leaves in a Hydroponic Cultivation System

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Introduction: The potential cause of pathogen contamination of edible portions of leafy greens has been considered as a result of direct contact with the pathogen through contaminated irrigation water and/or soil and as uptake from roots system through the vascular system. However, few comparative studies for both contamination routes have been conducted.

Purpose: We aimed to determine the principal route of pathogen contamination of the edible portion of leafy greens using a hydroponic system. Furthermore, the probability of the contamination of leaves affecting the contamination routes (form roots or leaves), inoculum levels and kind of pathogens were analyzed.

Methods: The contamination route (from roots or leaves) of pathogens to spinach leaves was investigated using a hydroponic cultivation system. Three major bacterial pathogens, *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* were inoculated into the hydroponic solution in which the spinach was grown to give concentrations of 10^6 and 10^3 CFU/ml. In parallel, the pathogens were inoculated onto the growing leaf surface via pipette to give concentrations of 10^6 and 10^3 CFU/leaf. The probability of the contamination of leaves affecting the contamination routes (from roots or leaves), inoculum levels and kind of pathogens was analyzed using logistic regression.

Results: While contamination was observed at a high rate through the roots system at higher inoculum levels (10^6 CFU) for all the pathogen tested, at lower inoculum levels (10^3 CFU)contamination was rare for all the pathogens. In contrast, the contamination through the leaf showed a very low rate even if the inoculum level was high. The probability of contamination was promoted through the roots and with higher inoculum levels in all pathogens tested in the present study. The result of logistic regression suggested that the risk of contamination via roots was 6.93 times higher than that via leaves.

Significance: The results in the present study indicated that the main route of the pathogen contamination of the growing spinach leaves in hydroponics was from the plant's roots rather than from contamination of the leaf itself.

P2-72 Characterization of Root Uptake and Systemic Transport of *Salmonella enterica* sv. Typhimurium into Cantaloupe and Honeydew Vines and Fruit

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Introduction: Among melons, cantaloupes are most frequently implicated in outbreaks and surveillance-based recalls due to *Salmonella*. The California industry identified irrigation water quality as an uncharacterized risk in relation to root uptake and internalization of *Salmonella* into melon fruit.

Purpose: To determine whether root uptake of *S. enterica* results in systemic transport to fruit at high doses of applied inoculum.

Methods: Cantaloupe and Honeydew vines (n=150) grown in greenhouse or replicated clay-loam field plots were inoculated with >8 log CFU/ml of attenuated *S.* Typhimurium. Recovery from vines after 24 h and 7 days of inoculation was facilitated by enrichment augmented with rifampicin. Drenching roots of seedling and trellised vines was used in greenhouse tests. Field inoculations included furrow-irrigation and injection of contaminated water in subsurface drip lines. Full-slip cantaloupe and mature honeydews were harvested (n= 415) at Days 31, 38 and 48 after inoculation. Melons were surface sterilized with silver nitrate and the abscission zone core was analyzed for *Salmonella*. Quantification of *Salmonella* in bulk and rhizosphere soil was assessed. This field experiment was repeated twice in consecutive years.

Results: Systemic transport from roots to vine was observed to be infrequent and transient under greenhouse conditions. No internalization of *Salmonella* was detected in vine or fruit under field conditions for either contamination-exposure method. Transfer of furrow-inoculated *Salmonella* was not detectable across the soil bed nor within the rhizosphere and roots of developing vines.

Significance: Salmonella internalization from soil and vascular systemic transport to fruit is unlikely to occur from contaminated irrigation water at any level that might rationally be anticipated. While contamination of the external rind remains a concern for irrigation sources in melon production, results suggest a high pathogen threshold may be used in defining appropriate microbiological standards for melon irrigation in California and regions with similar practices.

P2-73 Persistence of Somatic and F-Specific Coliphages, Potential Indicators of Fecal Contamination, on Spinach Foliar Tissue

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Introduction: Recent outbreaks of foodborne illness have been linked to consumption of fresh leafy greens. *E. coli* is commonly used as the indicator microorganism for fecal contamination on leafy greens. However, alternative microorganisms, such as coliphages, may deserve consideration as indicators of fecal contamination on produce.

Purpose: The objective was to determine if the recovery of somatic and F-specific coliphages was possible from inoculated spinach plants, and which of three methods was most sensitive in their detection. The persistence of coliphages on spinach plants was also evaluated.

Methods: Somatic (phiX174) and F-specific (MS2) coliphages were prepared and spot-inoculated on 4-week old spinach plants in growth chambers at 10^5 PFU/plant. Foliar tissue was aseptically harvested and homogenized in either sterile water, sterile magnesium (SM) buffer or 3% beef extract (BE). A soft agar (0.60% LB agar) overlay method with *E. coli* host strains CN13 (for phiX174) and Famp (for MS2) was used to determine phage recovery from spinach plants five times over 20 days. On each day of analysis, three plants inoculated with each coliphage were homogenized in each diluent.

Results: On Day 0, MS2 recovery was 3.42 log PFU/plant. After 4 days, MS2 phage declined by 1.78 log PFU/plant to below the level of detection (1.65 log PFU/plant), but was recovered by enrichment for two weeks. phiX174 coliphages persisted on spinach plants for 20 days, declining by 2.22 log PFU/plant from 5.42 log PFU/plant on Day 0. All diluents recovered both coliphages from spinach. Overall, BE was most effective in recovering phiX174 from spinach leaves, with significantly ($P < 0.05$) larger titers recovered on Day 20 compared to either water or SM. Uninoculated plants did not contain coliphages.

Significance: The recovery of phiX174 and MS2 with BE after 20 days indicates the potential utility of coliphages as indicators of fecal contamination on leafy greens.

P2-74 Long-term Stability of Norovirus on Farm and Agriculturally-relevant Environments

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Introduction: Human norovirus (NoV) causes gastroenteritis worldwide and is associated with a number of produce-related outbreaks. The design of effective inactivation and prevention procedures requires the knowledge of virus survival in environments applicable to the production and processing of fresh produce.

Purpose: To evaluate the extent of NoV risk along the farm-to-fork continuum, the survival of Murine norovirus (MNV), a surrogate for human NoV, has been studied on stainless steel disks, soil and in bottled water for 42 days and on lettuce for 15 days in the lab. The study was then followed on the farm during one lettuce planting/harvest cycle for 4 weeks.

Methods: Known concentrations of MNV were spiked onto/into the test matrices at room temperature (RT) or on the farm. The recovery methods were validated. The reduction in virus infectivity and genomic copies were investigated by plaque assay and real-time/conventional RT-PCR, respectively.

Results: A one-log reduction in the virus titre was achieved after: 29.40 days in water; 4.25 days on lettuce; 14.65 days on stainless steel disks; 12.14 days on loamy soil and 11.70 days on sandy soil. For farm testing, infectious virus was recovered from both soil and lettuce on the day of inoculation. Although infectious virus was not recovered at later time points, the viral genomes were detected for up to four weeks.

Significance: Long-term persistence of NoV, under both lab and field conditions, provides valuable information for developing risk assessments and control procedures to limit the chances for NoV transmission in the food supply.

P2-75 Human Foodborne and Zoonotic Viruses Detected on Fresh Strawberries

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Introduction: Enteric viruses have been recognized as the leading cause of non-bacterial gastroenteritis and infectious hepatitis outbreaks around the world. Fruits, in particular berries, may act as a vehicle for viral agents when they are manipulated by infected food handlers or through the use of contaminated irrigation water.

Purpose: The aim of this study was to evaluate the presence of pathogenic and potentially zoonotic viruses on irrigated fresh strawberries at harvest time.

Methods: A field experiment, was conducted in the Laurentides region of the province of Québec (Canada) in 2009. Irrigations were performed on July 14th and 28th from an adjacent river. Irrigation water samples were taken during the 2nd irrigation. Composite samples of 10 strawberries were aseptically taken in all 16 plots before irrigation, as well as 1 hour after irrigation, and also on July 29 and 30th. The presence of human norovirus, rotavirus, Torque teno virus and porcine Hepatitis E virus was verified in all samples. Feline calicivirus (FCV) and Murine calicivirus (MNV), as a sample process control, were added to every sample prior the concentration and the extraction procedures.

Results: Human viruses such as Norovirus GI (26.6%), rotavirus (3.3%), Torque teno virus (3.3%) and porcine hepatitis E virus (1.7%) were detected on strawberry samples after irrigation by RT-PCR, real-time RT-PCR and some amplicons were sequenced. No virus was detected in any water samples. One plot was detected positive for viruses before irrigation and 7 plots were positives 1 hour after irrigation. MNV (88%) was more constantly detected than FCV (61%) in strawberry samples.

Significance: This study reported that viral contamination of fresh strawberries can occur directly at the farm before harvest but no link could be established between irrigation and the contamination. Other factors such as soil contamination and agricultural practices should be evaluated in future works..

P2-76 Persistence of Poultry Associated *Salmonella* spp. on Spinach Plants

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Introduction: Pre-harvest spinach contamination can occur via irrigation water and can influence the persistence of *Salmonella* on spinach leaves. *Salmonella* persistence on spinach plants should be evaluated as nearby poultry farms can be a critical source of contaminated water run-off.

Purpose: The purpose of this study was to compare the persistence of *Salmonella* on spinach leaves when contaminated with strains isolated from produce and poultry. Further, efficacy of the California Leafy Green Marketing Agreement (LGMA) irrigation water quality criteria applied to spinach plants was also evaluated

Methods: Five strain mixtures of *Salmonella*, poultry and produce isolates were cultivated in water extracts of dairy manure solids. The individual 3-week old spinach plants ('Tyee Fe" cultivar) grown in BL-2 growth chamber were spray inoculated every 2 week with ~ 3 ml water containing 2.12 (low) or 4.12 (high) log *Salmonella* per ml. Plants were also irrigated with potable water every week to maintain plant turgidity. Three replicates of each plant shoot were analyzed on Day 1 and weekly for up to 6 weeks for *Salmonella* populations by direct plating on XLT4 agar and MPN (enrichment in BPW followed by selective enrichment in tetrathionate broth and subsequent spot plating on XLT4 agar).

Results: Salmonella were undetectable (< 1.1 MPN/g) on spinach leaves throughout the study when irrigation water was contaminated at low level of poultry or produce isolates. However, *Salmonella* were recovered every week from spinach leaves (4-5 log MPN/g) when inoculated at high level. *Salmonella* persisted at significantly higher numbers on spinach plants when contaminated with produce isolates.

Significance: Longer persistence of *Salmonella* on spinach leaves when contaminated at a high inoculum level reinforces need for water quality standards such as the California LGMA. Comparative evaluation of contamination sources (irrigation water) should be studied at the field levels.

P2-77 Survival of *Escherichia coli* O157:H7, *Salmonella* and *Listeria* in Manduca Hornworm Frass-fed Tomato Leaves

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Introduction: Larval pests, like *Manduca*, can cause major chewing damage on tomato plants. Foodborne illness pathogens on leaves can be ingested by hornworms and potentially excreted in their frass (excrement). Larvae grow rapidly, thus even a few larvae produce copious quantities of frass, which has a high contact potential as it falls onto leaves and fruits in a tomato canopy. No data are available on survival and growth of foodborne pathogens ingested by *Manduca* and excreted as frass.

Purpose: Determine growth and survival of *E. coli* O157:H7, *Salmonella*, and *Listeria* in frass produced by *Manduca* that have ingested contaminated tomato leaves.

Methods: *Manduca* larvae were reared in a laboratory on synthetic diet to third instar stage. Cultures of nonpathogenic strains of *E. coli* O157:H7, *Salmonella*, and *Listeria* grown in TSB 24h at 37°C, were washed 2x in PBS. Individual larvae were placed in containers and fed once with 8 tomato leaf disks inoculated with multiple 10µL drops containing a total of 2.9-6.8 x 10^3 cells. After leaf disks were consumed, larvae were fed uninoculated disks, and frass was collected 2-3 times for four days, diluted in buffer, and spiral-plated onto SMAC for *E. coli*, XLT4 for *Salmonella*, and MOX for *Listeria*. Primary dilutions were enriched and plated onto these same media. Three separate experiments were conducted with 5, 10, or 20 individual larvae per inocula and PBS dosed disks as controls.

Results: Preliminary and replicated tests revealed that consistent results required use of 10-20 larvae/inoculation. *E. coli*, *Salmonella*, and *Listeria* survived *Manduca* gut passage and were excreted in frass when 2.9x10^3 cells were ingested, as evidenced by recovery in the insect frass. Significant ($P < 0.05$) increases, from 3 to 5 logs, occurred in *E. coli* cell densities, whereas *Salmonella* cell densities increased 1 to 3 logs, which, while significant, were less than those for *E. coli*. *Listeria* counts in frass were highly variable among different hornworms and remained at levels in the range of the inoculation. *E. coli* was the only one of the three types of bacteria to show an increased population during the four days of frass assay. *Salmonella* cell densities did not increase after Day 1.

Significance: Hornworms are capable of vectoring small to very significant quantities of *E. coli* O157:H7, *Salmonella*, and *Listeria* in their frass for several days after initial ingestion of contaminated leaves, thereby providing a means of disseminating these pathogens in tomato fields. Moisture from rainfall and condensation could aid survival and spread of pathogens from frass into the cracks and crevices of tomatoes, potentially increasing food safety risk.

P2-78 Epiphytic Survival of Shiga-toxigenic *Escherichia coli* O145 on Baby Spinach Plants

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Introduction: Non-O157 Shiga-toxigenic *E. coli* (STEC) have previously contaminated leafy greens. In early 2010, 26 confirmed illnesses were traced to a multi-state outbreak involving shredded romaine lettuce contaminated with *E. coli* O145, a STEC. It is possible that the O145 serotype may be suited for growth and/or survival on leafy green surfaces.

Purpose: This study examined the epiphytic survival of O145 STEC on the surfaces of baby spinach leaves.

Methods: An inoculum containing four isolates of nalidixic acid-resistant *E. coli* O145 were cultured in a diluted dairy manure slurry and spot inoculated onto two-week old baby spinach plants maintained in a growth chamber (14 h photoperiod at 18°C and 10 h at 13°C, 75% humidity). Immediately after inoculation (0 h), 19 h, 24 h and then daily from Days 2 through 8, the aerial tissue from plants (n = 3) was homogenized in 40ml buffered peptone water and *E. coli* O145 populations were enumerated by spiral plating onto MacConkey agar. On Days 2 through 8, three plants were harvested daily to determine *E. coli* O145 counts via standard 3-tube MPN analysis.

Results: At time 0, 6.20 log CFU/plant *E. coli* O145 were recovered from the plants. *E. coli* O145 populations declined by almost 4 log CFU after 19h to 2.33 log CFU/plant. After 24 h, *E. coli* O145 populations were 1.94 log CFU/plant, with recoveries on Days 2 and 3 of 1.15 log and 1.09 log MPN/plant, respectively. Subsequent analyses on days 4 through 8 did not recover *E. coli* O145 from spinach plants.

Significance: E. coli O145 declined rapidly on the epiphytic surface of baby spinach plants. Even when inoculated at 6 log CFU/plant, *E. coli* O145 populations did not survive for more than three days. Data suggest that *E. coli* O145 isolates do not appear to persist for durations longer than other STEC on spinach foliar surfaces.

P2-79 Growth and Survival of *Listeria monocytogenes* in Mango (*Mangifera indica* Linn) Pulp

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Introduction: Understanding the growth and survival of *Listeria monocytogenes* in mango pulp (*Mangifera indica* Linn. var. Palmer), at different times and temperatures will be helpful in developing effective interventions for the control of this pathogen in the fruit.

Purpose: To determine the growth and survival of *L. monocytogenes* in mango pulp at different temperatures and incubation times.

Methods: Fruits without any defects (peel ruptures, bruised areas) were used in the experiment. Pulp was aseptically removed and homogenized in a previously sterilized blender. Mango portions were inoculated with saline suspensions of the test organisms, yielding final populations of approximately 1,000 CFU/g. The inoculated pulps were incubated at four different temperatures (-20°C, 4°C, 10°C and 25°C) and incubation times. At each sampling time, 1 mL of fruit pulp was collected, serially diluted in peptone water and pour plate dispersed in TSA-YE (45°C). The plates were incubated at 30°C for 24 h and bacteria were then counted, with results being expressed in CFU/g. Uninoculated pulp controls were also analyzed to ensure the absence of any background microflora before and after the incubation time.

Results: Generation times of 2h and 13h were obtained for *L. monocytogenes* at temperatures of 25°C and 10°C respectively. Survival of *L. monocytogenes* was observed at 4°C and -20°C after 9 days and 16 months respectively.

Significance: The study indicates that *L. monocytogenes* can grow well in homogenized mango pulp at 25°C and that the low temperature of 10°C retards but does not stop the growth of this bacterium. If a previous contamination of mango pulp by *L. monocytogenes* occurs, the temperatures of 4°C and 10°C cannot be considered a safe method for keeping this fruit.

P2-80 Role of Curli Expression by *Escherichia coli* O157:H7 on the Cell's Ability to Attach to Spinach

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Introduction: Shiga-toxigenic *Escherichia coli* O157:H7 (STEC) outbreaks have been linked to consumption of fresh produce. Mechanisms of bacterial interaction with plant surfaces should be investigated to develop mitigation strategies. Cellular appendages such as curli fibers have been suggested to be involved in STEC persistence in fresh produce as these curli are critical in biofilm formation and adherence to animal cells.

Purpose: To determine the role of curli in attachment of STEC on spinach in natural production environment and marketplace.

Methods: The curli expression by wild-type STEC strain, EDL933, and deriving curli-deficient (*csgA* and *csgD*) and curli-restored (*csgA*(pCsgA)) mutants grown on TSB agar at 20, 26 and 37°C was determined by Congo red (CR) binding assay and Scanning Electron Microscopy (SEM). Spinach leaves were spot inoculated with 10^6 CFU of bacteria/g of leaf and incubated at 12, 26 and 37°C for 18 h. After incubation, leaves were washed three times in PBS and then homogenized by Polytron® to determine populations of strongly attached bacteria. Further, attachment was confirmed by Laser Scanning Confocal Microscopy (LSCM).

Results: STEC Δ*csgA* and Δ*csgD* mutants didn't express curli at 20, 26, and 37°C, whereas EDL933 and Δ*csgA*(pCsgA) developed a clear red phenotype on CR medium at 20 and 27°C. At 37°C curli expression by EDL933 on CR medium was less pronounced compared to

Δ*csgA*(pCsgA). SEM and LSMS analysis confirmed EDL933 and Δ*csgA*(pCsgA) cells expressing extracellular matrix containing curli fibres. When incubated at 12 and 26°C, Δ*csgA* and Δ*csgD* attached to spinach leaves at significantly ($P \leq 0.01$) lower numbers compared to EDL933 and Δ*csgA*(pCsgA). However, attached bacterial populations among strains weren't significant when leaves were incubated at 37°C.

Significance: Understanding the role of curli in STEC attachment and persistence in vegetal matrices will help developing intervention strategies to remove pathogens from fresh produce and thereby reduce future recalls.

P2-81 Influence of Cell Surface Properties on Attachment of *Escherichia coli* O157:H7 to Spinach and Lettuce Leaves

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Introduction: The attachment of bacteria on leafy greens surfaces is a complex process regulated by various characteristics of the cell surface, growth medium and substratum. The mechanism by which *E. coli* O157: H7 attaches to leafy greens is not well understood.

Purpose: This study investigated the effect of growth in tryptic soy broth (TSB), Luria broth base, miller(LB) and nutrient broth (NB) on the ability of *Escherichia coli* O157:H7 to attach to spinach and lettuce under different ionic environments. Surface charge, hydrophobicity, and the capsule characteristics were determined.

Methods: Spinach and lettuce leaves were inoculated with 9 log CFU/ml cells for 2 h at 4°C. Viability of cells was examined by plate counts at the surface and cut edge.

Results: Cells grown in TSB attached more to spinach than those grown in LB and NB. Addition of sodium and calcium ions to the attachment medium increase attachment of cells grown in LB and NB on spinach surfaces than those grown in TSB. Cells preferentially attached to the cut edge of spinach leaf in comparison with intact surface. Cells grown in TSB, LB, NB in attachment medium of sterile distilled water exhibited 1, 0.2 and 0.5 log greater attachment to spinach than lettuce, respectively. Lectin characterization indicated that capsules from cells grown in TSB contained more D-Mannose, α-Fucose than the capsules of cells grown in LB nad NB. The zeta potential of cells grown in LB was more negative than that of cells grown in TSB and LB. Cells grown in LB and NB were more hydrophobic than cells grown in TSB.

Significance: These findings indicate that that the greater surface hydrophilicity, less negative charge, and capsule containing more D-Mannose, α-Fucose of *E. coli* O157:H7 grown in TSB may combine to increase attachment to spinach leaves.

P2-82 Genetic Mechanisms of *Salmonella* Typhimurium Surface Attachment on Tomatoes

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Introduction: The rise of produce-linked salmonellosis outbreaks has directed attention towards the ecology of *Salmonella* spp. on plants. Aggregative fimbriae and cellulose production are important in the attachment of *Salmonella* spp. on plant seedlings. The transcriptional regulator in *Salmonella* spp., *agfD*, is thought to play a key role in the attachment to produce since AgfD regulates both aggregative fimbriae and cellulose production. The role of *agfD* in the attachment of *Salmonella* spp. to ripe produce, such as tomatoes, has yet to be determined.

Purpose: The aim was to study the role of *agfD* in the attachment of *S. enterica* serovar Typhimurium to the surface of tomatoes to further characterize the genetic basis for the surface interactions between *Salmonella* spp. and plants.

Methods: Wild-type *S.* Typhimurium and a kanamycin-resistant Δ*agfD S.* Typhimurium (MKF1) mutant were used in a side-by-side comparison study where the surface of tomatoes were inoculated with either MKF1 or wild-type *S.* Typhimurium. The tomatoes were rinsed with sterile DI water after two minutes and dried for one hour. The tomatoes underwent another rinsing and were placed in whirl-pack bags containing phosphate buffered saline (PBS) in which the tomatoes were rubbed by hand for one minute. The initial rinsate, secondary rinsate, and PBS solutions were plated onto xylose-lysine deoxycholate (XLD) agar plates to obtain population concentrations for the two strains.

Results: Populations of MKF1 and wild-type *S.* Typhimurium were recovered from the tomatoes after the initial rinse was performed, indicating that both cultures were able to primarily attach to the tomato surface. The wild-type *S.* Typhimurium was able to maintain higher population levels on the tomato surface than the MKF1 strain.

Significance: The results support the importance of aggregative fimbriae and cellulose as well as the role of AgfD in the attachment of *S.* Typhimurium to the surface of tomatoes.

P2-83 Packaging and Seasonal Effects on Coliform Contamination of Romaine lettuce, *Lactuca sativa* var. *longifolia*

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Introduction: Investigations of bacterial contamination in lettuce have largely focused on lettuce in fields and processing plants, to determine the sources of contamination. This is certainly important, but may not reflect the contamination in lettuce available to consumers. Studies investigating leafy greens at the commercial level have focused on ready-to-eat salads (RTES), but not other packaging of lettuces.

Purpose: We investigated bacterial contamination in commercially available Romaine lettuce in Western Pennsylvania. Lettuces were analyzed by the MPN method, and we statistically analyzed the results against several factors including packaging, season, store, brand, and freshness.

Methods: For 53 consecutive weeks beginning June 2009, we purchased Romaine lettuce samples, from 7 different stores, packaged three different ways: bare heads of Romaine (under sprayers); bagged hearts of Romaine, packaged two or three to a bag and sealed; and Romaine RTES. Most Probable Number (MPN) analysis was performed with 25 g lettuce samples, mixed by a stomacher. Lactose-fermenting subcultures were streaked onto Endo agar to assess probability of coliform identity.

Results: There were significant differences ($P < 0.0001$) in the MPN due to packaging, with hearts having the lowest MPN, and RTE salads having the highest. There was some variation by store for bare heads, but not for hearts or RTES. Different brands of RTES did not have statistically different MPNs. Bacterial load increased as RTES approached its "sell by" date ($P = 0.003$). Bare lettuce MPN showed a seasonal trend, with summer months higher MPNs than winter months, but hearts did not show this trend, and RTES showed an overall increase in MPN throughout the study. Few of the samples testing positive for MPN produced dark colonies on Endo agar, suggesting that most of the lactose-fermenting bacteria are not necessarily coliforms.

Significance: While several factors play a role in the measured contamination levels, our results suggest that packaging may be the most important of those factors. These results may be of interest not only to consumers, but to producers and stores in assessing the quality of leafy produce.

P2-84 Survival and Epidemiology of *Escherichia coli* on Diverse Fresh-cut Baby Leafy Greens under Model Pre-harvest to Post-harvest Conditions

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Introduction: Prevention and sanitation are important elements for managing microbial quality and safety of fresh-cut produce. The limitation of the post-harvest washing to remove and disinfect attached microorganisms with sanitizers remains a priority for discovery. For leafy greens, bacterial attachment is variable and acutely influenced by physicochemical interactions, leaf structure, and temporal factors.

Purpose: To model *Escherichia coli* survival on different leafy greens from production through simulated post-harvest handling.

Methods: Mizuna, Red Chard and Tatsoi leaves were grown under greenhouse conditions. A cocktail of generic *E. coli* and attenuated *E. coli* O157:H7 (attO157) were spray-inoculated separately (log 4.2 CFU/cm^2). Leaves were harvested as mini-greens at commercial maturity, processed in a model washing system treated with chlorine dioxide (3 mg L-1, contact time 90s) and stored for 7 days at 5°C under MAP. Recovery and detection of generic *E. coli* and attO157 was conducted after 2, 7 and 10 days post-inoculation, following disinfection, and 7 days post-washing. For att O157, detection by qRT-PCR was used for populations declining below the limit of direct-plating or filtration enumeration. Commensal *E. coli* differentiation was carried out using REP-PCR.

Results: Significant variability of attached generic *E. coli* and att O157 was observed depending on the leafy vegetable. Generic *E. coli* was detected on tatsoi leaves throughout the post-harvest period, while dropping below the limit of detection for other types. After storage, all the samples were positive following enrichment. Attenuated *E. coli* O157:H7 was not recovered by direct plating after 7 days post-inoculation. Molecular analysis confirmed the sporadic presence of *E. coli* O157:H7 below the limit of detection at all subsequent time-points.

Significance: Heterogeneous low populations of generic and attenuated *E. coli* could survive from production to commercial distribution. Permissible doses of chlorine dioxide could not fully disinfect either non-pathogenic surrogate from the leaves under test conditions.

P2-85 Modeling the Variability of Growth Rate and Lag Time among Different Strains of *Salmonella* and *Listeria monocytogenes* in Minimally Processed Lettuces

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Introduction: Salmonella and *L. monocytogenes* are pathogens of concern for minimally processed vegetable safety and they have been isolated from lettuce, the most commonly consumed vegetable in Brazil.

Purpose: The purpose of our research was to model the effects of temperature on the growth rate (μ) and lag times (λ) of three different strains each of *Salmonella* spp. and *L. monocytogenes* in a mixture of shredded iceberg and crisp lettuce.

Methods: Sanitized shredded lettuce was inoculated by dipping in suspensions of three different *Salmonella* (*S.* Typhimurium #: 277, 386 and *S.* Enteritidis ATCC 13076) and *L. monocytogenes* (4b #: 413, 494 and 581) strains. Those strains with three digit numbers were isolated from Brazilian produce. After centrifugation and packaging (5% CO_2, 15%O_2 and 80%N_2), sealed packs were stored at six temperatures (7–30°C) for different time intervals and *Salmonella* and *L. monocytogenes* were enumerated using MLCB and Oxford agars, respectively. Primary growth parameters (μ and λ) were determined using the Baranyi model with the software DMFit 2.1 and the Ratkowsky (square root) model was used to describe μ and λ as a function of temperature.

Results: Correlation coefficients for every primary growth curves and secondary models were good, and always >0.9. Estimations for μ varied between 4-17% and 11-47% for the different *Salmonella* and *L. monocytogenes* strains studied, respectively. The parameter λ varied between 6-41% and 11-40% for the same pathogens. In the secondary modeling, μ showed a linear trend when regressing temperature versus square root of this parameter (r^2 = 0.95 and 0.98 for *Salmonella* and *Listeria*, respectively). For λ estimation, a natural logarithmic transformation provided the best fit, with similarly good r^2 = 0.95 for both for *Salmonella* and *Listeria*.

Significance: Our findings will be useful for the development of risk assessment models to estimate the probabilities of salmonellosis and listeriosis due to the consumption of MPV in Brazil.

P2-86 Selection and Characterization of DNA Aptamers with Binding Specificity for *Listeria* spp.

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Introduction: Nucleic acid aptamers show advantages over traditional capture ligands (such as antibodies), including reduced cost, ease of production and modification, and improved stability. Aptamers are being investigated for selective concentration and purification of foodborne pathogens from complex sample matrices such as foods.

Purpose: To identify DNA aptamers with binding specificity to *Listeria* spp.

Methods: A carboxyfluorescein (FAM)-labeled single stranded DNA library was exposed to *L. monocytogenes* cells in log phase (6 h cultures) or stationary phase (12 h cultures) and aptamers with binding selectivity were selected using the SELEX (Systematic Evolution of Ligands by Exponential enrichment) method. Six SELEX rounds were followed by two counter-SELEX rounds against a cocktail of non-*Listeria* spp. Aptamers with binding selectively to *L. monocytogenes* were separated, sequenced, and characterized by flow cytometry.

Results: One aptamer each isolated after SELEX applied to log phase (aptamer LM-6-02) and stationary phase (aptamer LM-12-06) *L. monocytogenes* cells was chosen for further characterization. Initial flow cytometry analysis suggested relatively high binding efficiencies [45-60% of *L. monocytogenes* target cells (n = 100,000) fluorescent using 500 pmoles] for both aptamers. Dissociation constants (Kd values) of 2.1±0.5 and 1.1 ± 0.1 μM were obtained for aptamers LM-6-02 and LM-12-06, respectively. Interestingly, even though aptamers were selected for using *L. monocytogenes*, they demonstrated similarly high binding efficiencies (41-64%) for both *L. seeligeri* and *L. grayi*. Aptamer binding exclusivity analysis showed low apparent cross-reactivity with other foodborne bacteria, including *E. coli* O157:H7, *Salmonella* spp. and *Brochothrix thermosphacta* for which binding efficiencies never exceeded 10%. Minimal cross-reactivity was observed for *Bacillus cereus* (13-18% cells fluorescent).

Significance: The advantage of applying SELEX to cells in different growth phases lies in the opportunity to produce aptamers with broader reactivity, since cell surface moieties expressed at all growth phases can serve as binding targets. Aptamer candidates LM-6-02 and LM-12-06 are currently being evaluated for synergistic capture of *Listeria* spp. from complex sample matrices for detection by PCR.

P2-87 DNA Extraction Procedures for Real-time PCR Detection of *Listeria monocytogenes* and *Listeria* spp. from Artificially Contaminated Food Samples

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Introduction: Identification of *Listeria* species and specifically *Listeria monocytogenes* by real-time PCR allows rapid detection of the pathogen from contaminated food.

Purpose: The objective of the current research is to evaluate DNA extraction protocols from food enrichments for detection of *Listeria monocytogenes* by two real-time PCR procedures.

Methods: Four DNA extraction protocols from Buffered Listeria Enrichment Broth and DemiFraser/Fraser enrichments of artificially contaminated foods (Asadero cheese, Queso Fresco, Brie cheese, guacamole, coleslaw, and smoked salmon) were evaluated with two real-time PCR procedures for detection of *Listeria monocytogenes* and *Listeria* spp. For DNA extraction, a semi-automated magnetic particle-based extraction instrument (MagNA Pure Compact), with and without enzymatic treatment, PrepSEQ™ Rapid Spin columns and wash-spin-boil (WSB) sample preparation techniques were compared. Real-time PCR analysis of the DNA extracts were conducted on the ABI 7500 fast platform using a multiplex *Listeria* spp./*L. monocytogenes* real-time (LIS) PCR assay targeting regions of the *iap* gene and designed to simultaneously detect *L. monocytogenes* as well as all *Listeria* species and the MicroSeq® *Listeria monocytogenes* Pathogen Detection kit.

Results: MagNa Pure Compact DNA extraction with or without enzymatic digestion of the samples was effective for template preparation from BLEB and Fraser Broth enrichments for both PCR procedures for all of the foods tested. The rapid spin columns did not work well with most food samples, causing numerous false-positive reactions in uninoculated food samples (16-100%) and inhibition of DNA amplification particularly in Fraser broth enrichments. A 1:10 dilution of the template improved its performance with the LIS assay but not the MicroSeq® *Listeria monocytogenes* assay. The WSB preparations worked well with the LIS PCR assay, but even 1:10 dilutions of the preparations gave poor results with the MicroSeq® *Listeria monocytogenes* assay for most foods.

Significance: These results show that template preparation is important for reliable real-time PCR screening of food enrichments for *Listeria monocytogenes*. The MagNa Pure Compact procedure without additional enzyme treatment of the sample resulted in template preparations that were suitable for both PCR assays. The LIS PCR assay was the most reliable test for detection of *Listeria* spp./*L. monocytogenes* from both BLEB and Fraser broth food enrichments.

P2-88 Development and Initial Evaluations of Three Scorpion™ Probe-based Multiplex Real-time PCR Assays for the Detection of Six Shiga Toxin-producing *Escherichia coli* (STEC) Serogroups, *E. coli* O157:H7, and the *eae*, stx_1 and stx_2 Genes

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Introduction: In addition to continued testing for *E. coli* O157:H7, new regulations in the United States will soon require industry to begin monitoring for six Shiga toxin-producing *E. coli* (STEC) serogroups. Initially, it is anticipated that the regulations will specifically require detection of the so called "big-six" STEC (O26, O45, O103, O111, O121, and O145) as these have been most frequently associated with outbreaks of foodborne illnesses in the United States. There may be an additional requirement related to the detection of the virulence genes *eae*, stx_1, and stx_2 though it is still unclear if/how this proposed regulation will be implemented. Not withstanding the final regulation, there is a desire to couple detection of the big six STEC with virulence gene detection or use virulence gene detection alone as a monitoring tool.

Purpose: The purpose of this study was to determine the feasibility and conduct initial evaluations of three separate multiplex Scorpion™ probe-based PCR assays for the detection of the big-six serotypes of STEC, *E. coli* O157:H7, and the *eae*, stx_1, and stx_2 genes.

Methods: The three multiplex assay configurations were as follows: Assay 1 - O26, O111, O121, and internal positive control (IPC); Assay 2 - O45, O103, O145, IPC; Assay 3 - O157:H7, *eae*, stx_1, stx_2 and IPC. Studies evaluating the sensitivity of each of the new real-time assays were conducted using titrations of cell lysates. Cell lysates were also used to conduct initial inclusivity and exclusivity studies for each assay configuration.

Results: Sensitivity of each assay with cell titers in TSB for each of the various PCR targets was shown to be $\leq 1.0 \times 10^4$ CFU/mL. Each assay was shown to be 100% inclusive for the strains tested (n = 20–50 per assay). Moreover, no cross-reactivity with closely related strains in any of the assays was seen.

Significance: These results demonstrate the feasibility of deploying a panel of three novel real-time PCR assay configurations for the detection and monitoring of STEC O groups including O157:H7 as well as the virulence genes, *eae*, stx_1, and stx_2. The approach demonstrated could easily be expanded to include additional multiplex assays should regulations continue to expand into other O groups or virulence gene markers.

P2-89 Primers with 5'AT-rich Flap Increases the Sensitivity of PCR for the Detection of *Escherichia coli* O157:H7

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Introduction: Escherichia coli O157:H7, a major foodborne pathogen, poses a serious threat to human health. Early detection of the pathogen in food, environmental and clinical samples is critical to managing human health concerns.

Purpose: The aim of this study was to evaluate the effect, on PCR sensitivity, of adding a 5' AT-rich overhanging sequence (flap) to the design of primers specific for the detection of *E. coli* O157:H7.

Methods: Specific primers targeting the *rfb*E O157 gene were synthesized with or without a 12-bp 5' AT-rich overhanging sequence. PCR sensitivity assays were conducted using purified *E. coli* O157:H7 genomic DNA, crude cell lysates, and genomic DNA/ crude cell lysates spiked with DNA extracted from tomato and jalapeno peppers surface washes. PCR amplicons from three replications were eluted and quantified using nano drop spectrophotometer.

Results: Amplicon band intensity was significantly greater when primers contained the flap, and the yield of PCR amplification was greater by 23%.

Significance: Improvement in the efficiency of PCR yield has potential applications in foodborne disease epidemiology and management, and in biosecurity and microbial forensics applications, as fewer target pathogens can be detected in less time.

P2-90 Equivalence of PCR and Phage Ligand Assay for the Detection of *Escherichia coli* O157:H7 in Ground Beef

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Introduction: Regulatory requirements and implementation of test and hold sampling plans have increased the need for rapid and effective test methods for the detection of *E. coli* O157:H7 in ground beef.

Purpose: The objective of this study was to compare the performance of a new automated next-day method to commercial PCR assays for the detection of *E. coli* O157:H7 in ground beef. The VIDAS® UP *E. coli* O157 assay was compared to the DuPont Qualicon BAX® as used by FSIS and BioControl GDSTM systems.

Methods: Ground beef samples inoculated with 2–5 cells of *E. coli* O157:H7 were enriched in buffered peptone water supplemented with vancomycin for the phage ligand assay (VIDAS UP *E. coli* O157), in modified TSB for the USDA/BAX assay, and in modified EHEC for the GDS assay. All broths were incubated at 41.5°C for 8 – 10 h. Twenty inoculated and five uninoculated samples were tested for each method for two separate replications using two separate strains of *E. coli* O157:H7.

Results: E. coli O157:H7 was not detected in the uninoculated samples using VIDAS UP *E. coli* O157. When the data from two replicates were combined, 22, 27, and 19 of the 40 inoculated samples screened positive samples after 8 h incubation and 34, 29, and 23 screened positive samples after 10 hr incubation, from the VIDAS UP, USDA/BAX, and GDS methods, respectively. Two of the screened positive samples for the FSIS/BAX were determined to be false positives.

Significance: For all methods, more screened positives were detected from the 10 hr enrichment than from the 8 h enrichment for one of the *E. coli* O157:H7 isolates. Therefore, a minimum of 10 hr enrichment would be recommended for 325 – 375 g ground beef samples. VIDAS UP *E. coli* O157 was equivalent to the two other test methods for the detection of *E. coli* O157:H7 following 10 h of incubation.

P2-91 A Rapid Molecular Method for the Detection of *Escherichia coli* O157 and *Salmonella* in Foods Using Loop-mediated Amplification (LAMP)

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Introduction: Rapid detection of *Escherichia coli* O157 and *Salmonella* spp. is essential for food safety. To accelerate the process and to test for both microbes from the same food sample, a rapid co-enrichment procedure was developed for use with the isothermal loop-mediated amplification (LAMP) method for detection from foods within 8 h. In addition, an overnight enrichment method for composited ground beef samples was developed for use with LAMP, giving a time-to-result for large samples in less than 24 h.

Purpose: This method comparison study is to evaluate the Loopamp® kits for the detection of both *E. coli* O157 and *Salmonella* spp. from intentionally co-contaminated foods (ground beef, beef trim, spinach and lettuce), comparing to the relevant USDA or FDA reference methods. *Salmonella* detection from intentionally-contaminated ground turkey and chicken rinsates will also be tested and compared to the USDA method.

Methods: For testing from ground beef, beef trim, spinach and lettuce, the food matrix was bulk-inoculated with both organisms and subjected to cold stress for 48-72 hr. A low-dose inoculum was used in order to achieve a fractionally positive detection rate. Twenty samples (25 g for beef, 200 g for leafy greens) were enriched for 6–7 h at 42°C and then sampled for LAMP testing, while parallel sets of samples were tested by the USDA (beef) or FDA (produce) method. Larger 375 g ground beef samples with a low-dose inoculum were tested using an overnight enrichment culture followed by LAMP testing. For additional *Salmonella* testing, ground turkey was bulk-inoculated and chicken carcasses were individually inoculated with *Salmonella* only. After cold-stress, 25 g turkey samples and 30 mL of chicken rinsates were cultured for 6–7 h enrichment and then sampled for *Salmonella* LAMP testing. Parallel samples were tested by the USDA protocol. Results were compared using the Mantel-Haenszel chi-square method for unpaired samples.

Results: This investigation is our AOAC Performance Tested Method validation to be completed in late spring 2011. The final results will be presented at the meeting.

Significance: The LAMP method compares favorably in sensitivity to the USDA and FDA reference methods and provides a rapid procedure to detect *E. coli* O157 and *Salmonella* from foods.

P2-92 Comparison of Five Pre-enrichment Media for the Recovery of *Salmonella* from Leafy Green Vegetables with Real-time PCR Methods

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Introduction: Leafy greens are among the top 10 foods which have been responsible for the largest number of food poisoning illness since 1990, according to a list recently released by the FDA. In the past several years, *Salmonella* outbreaks from fresh leafy greens have become an increasing problem.

Purpose: To determine the relative efficacies of five pre-enrichment media for the recovery of *Salmonella* from artificially contaminated leafy greens: lactose broth (LB; current BAM *Salmonella* pre-enrichment for leafy green vegetables), buffered peptone water (BPW), modified BPW (mBPW), universal pre-enrichment broth (UPB) and BAX broth.

Methods: Four *Salmonella* serovars were used as inocula. Fresh leafy greens such as baby spinach, Romaine lettuce and Italian parsley were spray inoculated and stored under refrigeration for 3 days before analysis. The inoculation level (0.015 ~ 0.35 CFU/g) was determined by the most probable number method. Twenty-five g leafy greens were soaked in 225 mL portions of LB, BPW, mBPW, UPB and BAX broth. The pre-enrichments were incubated for 24 h at 35°C. The BAM *Salmonella* culture method was followed thereafter. Two Real-time PCR analyses were performed on 24 h-incubated pre-enrichment broths on three Real-time PCR detection systems: BioRad CFX 96, Cepheid SmartCycler and ABI Fast 7500.

Results: qPCR tests show that four pre-enrichment media: BPW, mBPW, UPB and BAX were significantly more productive ($P < 0.05$) than LB for the recovery of *Salmonella* from artificially contaminated leafy greens after 24 h pre-enrichment. BAM culture analysis show no significant differences among five pre-enrichment media ($P > 0.05$) for the recovery of *Salmonella* from these produce types.

Significance: This study investigated the potential need for the development of an improved BAM *Salmonella* pre-enrichment method for the detection of *Salmonella* from leafy green vegetables. Real-time PCR test can be used as an effectively rapid screening tool.

P2-93 Detecting *Salmonella* in 375-g Pet Food Samples with Alternative Media

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Introduction: Dry pet food contaminated with *Salmonella* can pose a significant health risk to humans, especially young children, through the improper handling of pet food or contaminated excrement. Developing effective testing methods for detecting *Salmonella* contamination in such products will provide significant benefits by safeguarding public health and improving product quality.

Purpose: To evaluate and compare the performance of a PCR-based assay on dry pet food enriched in various media (LB, BPW, MP) through feasibility and confirmation studies.

Methods: For feasibility studies, samples were homogenized 1:10 in LB, BPW or MP media, allowed to stand for 60 minutes at room temperature, then mixed before incubation. Samples enriched in LB or BPW were incubated at 35°C for 22–26 hours; those enriched in MP media were incubated at 42°C for 22–24 hours. Aliquots were removed pre- and post-regrowth (enriched samples diluted 1:50 in BHI broth and incubated at 37°C for 3 hours) for testing with the test method. For culture comparison study, samples were enriched in either BPW or MP media as in the feasibility studies. The pH left at room temperature for 60 minutes; no adjustments of all samples was checked before and after samples were necessary. After enrichment, samples were tested with the modified FDA-BAM culture method, or diluted and regrown before testing with the test method.

Results: For 25-g spiked samples with or without regrowth, LB and BPW returned 1 out of 3 positive results, while MP media returned 3 out of 3 positive results. For 375-g spiked samples, LB with or without regrowth returned 0 out of 3 positive results. MP media without regrowth returned 0 out of 3 positive results, and MP media with regrowth returned 1 out of 3 positive results. For 375-g spiked samples enriched in BPW and MP media, both with regrowth, there was no difference in results between the test method and the reference culture method. For all studies, results between the two *Salmonella* PCR assays were identical.

Significance: The results of these studies demonstrate that, when used with the BAX® System: 1. MP media, with or without regrowth, returns results equivalent to or better than both LB and BPW as enrichment for detecting *Salmonella* in 25-g samples of dry pet food; 2. BPW and MP media, when used with regrowth, are effective enrichment media that do not require pH adjustment for detecting *Salmonella* in 375-g pet food samples; 3. Additional studies will be performed to compare and verify the media performance of LB to the MP and BPW.

P2-94 Development of Oligo-conjugated Nanoparticle-based Methods for Rapid, Sensitive and Cost-effective Detection of Foodborne Pathogens

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Introduction: Any pathogen contamination of our food and water chain can pose a serious threat to human health. In order to effectively safeguard consumers, foods need to be tested using methods with greater sensitivity and speed at lower costs than current methods. While existing molecular methods, such as PCR, are increasingly used in food testing, they have limitations. New approaches are needed to extend the limits of current molecular methods.

Purpose: To develop a rapid, sensitive and low-cost approach for detection of foodborne pathogens based on nanoparticle (NP) technology and a novel low-cost fluorescence reader.

Methods: Capture and detector DNA probes specific to sequences of *Salmonella* species and *Listeria monocytogenes* were conjugated to magnetic beads and fluorescent NPs (FNPs), respectively. The oligo-conjugated magnetic beads and FNPs were used to hybridize the specific target sequences in crude sample lysates, allowing magnetic separation of the targets from the samples and direct detection of the FNPs' signals by a fluorescence reader. A combination of tools/methods was used to evaluate the oligo-NP assays, including a novel prototype fluorescence reader, a fluorescence microscope and real-time PCR assays.

Results: The oligo-NP assays were found to be specific when evaluated with 21 *Salmonella*, 22 *L. monocytogenes* and 85 non-target bacteria strains. The assays were able to detect 1-5 CFU/25 g or 25 mL food sample after pre-enrichment when chicken, milk and pork were inoculated with *Salmonella*, and when vegetable mix, sausage and potato & egg salad were inoculated with *L. monocytogenes*. The methods were able to detect ~10^4-10^5 CFU/mL in pre-enriched food samples post-spiked with the target organisms. The methods were also evaluated by testing enrichment cultures of 10 poultry swab samples for *Salmonella* and 16 ice cream, cheese, and meat samples for *L. monocytogenes*, and showed higher sensitivity than the conventional culture methods. The oligo-NP procedures can be completed within 30 minutes after sample enrichment.

Significance: The oligo-NP-based approach can potentially lead to commercially valuable diagnostic test methods for more rapid, sensitive and cost-effective detection of foodborne pathogens. This will provide a valuable tool for the implementation of HACCP and quality assurance programs in our food chain to minimize foodborne outbreaks.

P2-95 Development of a Multiplex PCR Method for Detection of Bacterial Spoilage Organisms in Leafy Greens

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Introduction: Spoilage microorganisms on leafy greens are soil inhabitants that are introduced to the crop from seed and can persist throughout their growth in the field, during harvesting and post-harvest handling, or even during storage and distribution. Soft rot diseases are the major cause of spoilage in crops worldwide, many due to *Erwinia carotovora (Pectobacterium carotovorum)*, a gram negative phytopathogenic bacterium. The bacteria can be dispersed through the harvesting and handling equipment for leafy greens, in the storage facility, and on contact surfaces along the distribution chain.

Purpose: To develop a multiplex PCR method for detecting spoilage microorganisms on enriched leafy greens using *hrp* (hypersensitive and pathogenicity response) -*hrc* (hypersensitive response, pathogenicity, and conserved) gene clusters, and cell wall degrading, pectolytic enzyme genes.

Methods: A total of 180 enriched-leafy green samples, collected in Salinas, CA, were randomly chosen for the test using specific primers designed from the *hrp-hrc* and enzyme target genes of pathogenic phytobacteria namely, *E. carotovora, E. amylovora, Pseudomonas syringae*, and *Xanthomonas campestris*. Specific primers were also designed from lytic enzymes of *P. putida*, and *P. fluorescens*. The resulting PCR amplicons were resolved by 2% agarose gel electrophoresis containing ethidium bromide.

Results: Of the 180 samples, 67 (37.2%) were shown to harbor both *E. carotovora* and *P. syringae*, the most prevalent plant pathogens causing spoilage in the leafy green samples; 46 (25.5%) revealed either *E. carotovora* or *P. syringae*; 2 (1.1%) had all four major plant pathogens. Sixty-four (35.5%) of the samples displayed no incidence of any of these spoilage microorganisms.

Significance: The presence of spoilage microorganisms in food generally indicates shorter shelf life, hence potential financial losses. Early detection of their presence will aid in an appropriate post-harvest management strategy which can extend the shelf life of food and reduce product loss.

P2-96 Rapid Multiplex Identification of Foodborne Bacterial Pathogens via PCR/MS on the Plex-ID System

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Introduction: Traditional culturing methods of characterizing foodborne bacterial pathogens can take weeks to provide an identification at the serotype level. Novel molecular methods of characterization can provide results within hours of DNA extraction. One emerging technology for identification of foodborne bacterial pathogens utilizes broad PCR amplification coupled to mass spectrometry to allow for simultaneous characterization of multiple pathogens on the same assay plate.

Purpose: The objective of this study was to evaluate the capabilities of a novel technique, utilizing the Plex-ID system, for the identification and characterization of foodborne bacterial pathogens.

Methods: DNA from samples in the pathogen collection at PRL-SW was analyzed including DNA from *Salmonella enterica* isolates, isolates of EHEC (both O157:H7 and non O157), and isolates of *Shigella* and EIEC. After DNA extraction, all stages of sample preparation and analysis, from PCR through desalting and mass spectrometric analysis, were performed on the same plate reducing the possibility of contamination and sample loss.

Results: Of common disease causing *S. enterica* serotypes, 19 of 29 were correctly identified, and *E. coli* O157 was consistently given the correct serotype (66 of 67). However, serotyping was less reliable with other isolates. Only 9 of 71 less common *S. enterica* were correctly identified, and non-O157 *E. coli* (7 of 16) and *Shigella* and EIECs (8 of 47) had similarly low rates of identification.

Significance: This study indicates that combining PCR and mass spectrometry has potential as a rapid, sensitive method of identifying foodborne bacterial pathogens down to the serotype level with the possibility of vastly improving the capabilities of public health and regulatory laboratories to quickly identify bacterial pathogens. Future advances in primer design and in the reference database, which are currently in development, will improve the breadth of the instrument's characterization capabilities.

P2-97 Verification of the First Automated Sample Preparation and PCR Setup Robotic Workstation for the Food Industry

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Introduction: Most *Salmonella* infections are caused by ingestion of contaminated food. For laboratories performing high-throughput routine testing for foodborne pathogens, automation is key to greater efficiency. Advantages of automating sample preparation and PCR setup include traceability, data security, accuracy and reproducibility.

Purpose: Together with an international confectionery manufacturer, BIOTECON Diagnostics designed and performed verification and validation testing on an automated system, the foodproof® RoboPrep+ Series robotic workstation, for sample preparation and PCR setup.

Methods: Automatic DNA isolation was performed on the foodproof® RoboPrep+ Series robotic workstation using the new foodproof® Magnetic Preparation Kit I for automatic DNA isolation. Real-time PCR was then automatically setup by the foodproof® RoboPrep+ Series robotic workstation using the isolated DNA and foodproof® *Salmonella* Detection Kit for real-time PCR. Real-time PCR results were then evaluated. This verification study of the foodproof® RoboPrep+ Series robotic workstation involved testing specificity, sensitivity, robustness, and cross-contamination risk.

Results: The time necessary to process 96 samples on the foodproof® RoboPrep+ Series robotic workstation at one time was 2 h 20 min for DNA isolation and 20 min for real-time PCR setup for a total of 2 h and 40 min. Thirty-one *Salmonella* serovars and strains were tested for specificity with all being detected. Sensitivity of the method with 42 different food samples resulted in a detection limit between 5.0×10^3 CFU/ml and 5.0×10^4 CFU/ml for all matrices. Robustness testing of abnormally high food sample matrix compared to enrichment broth (1:7) showed no inhibition of *Salmonella* detection for all food samples tested. Studies found no cross-contamination to occur during tests with random placement of 48 *Salmonella* positive and 48 *Salmonella* negative samples.

Significance: The foodproof® RoboPrep+ Series robotic workstation is the first robot able to automatically perform DNA isolation and real-time PCR setup designed specifically for the food industry. These intensive internal validation studies of the foodproof® RoboPrep+ Series robotic workstation and foodproof® Magnetic Preparation Kit I coupled with the foodproof® *Salmonella* Detection Kit demonstrate this is a highly specific, sensitive, and robust system. The effectiveness and capability of the robotic workstation to automatically isolate highly-purified DNA and set up PCR is able to provide laboratories accurate, precise, as well as reproducible results.

P2-98 Towards Improved Detection of *Cryptosporidium* on Fresh Produce

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Introduction: Cryptosporidium has been found contaminating a variety of fresh produce worldwide. Current detection methods lack sensitivity and specificity for this parasite. Furthermore, because this protozoan cannot be cultured like bacteria to produce large numbers that facilitate detection, optimum recovery from vegetal matrices is essential for detection. Based on current recovery and detection methods, data on the extent of parasite foodborne contamination is woefully inadequate.

Purpose: Develop improved washing-recovery methods to facilitate detection of contaminated produce.

Methods: Spinach leaves were spot-inoculated with 2.7×10^6 *C. parvum* oocysts /leaf, air dried and incubated overnight at 4°C. Recovery of oocysts from spinach was conducted on a gyratory shaker for 30 min in 50-mL tubes (the seeded side of the leaf facing internally in the tube) in 45 ml of Alconox® 0.1% or water. This washing procedure was repeated twice. Recovered oocysts were immuno-stained with MeriFluor reagent and counted. All inoculation sites were excised from the washed leaves and subjected to nested PCR analysis to determine the presence of *C. parvum* DNA.

Results: Most oocysts were released with the first washing. The second wash moderately, but significantly ($P \leq 0.05$), improved recovery rates. The addition of 0.1% Alconox® greatly increased the percentage of recovery from 53.7 ± 7.2% in water to 73.6 ± 3.2% in Alconox® solution. All contaminated sites on the leaves were positive for *C. parvum* DNA, suggesting that in spite of the improved recovery some oocysts still were not removed from vegetal matrices. This fact should be taken into consideration by fresh produce processors' washing criteria.

Significance: Development and application of a superior recovery procedure will enable a much more accurate determination of the extent of contamination of fresh produce and reduce/prevent future foodborne outbreaks of cryptosporidiosis. Potentially these findings can be applicable to other protozoan pathogens that strongly adhere to fresh produce.

P2-99 A Real-time RT-PCR Method to Discriminate Infectious from Non-infectious Norovirus Strains

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Introduction: The inability of molecular amplification to discriminate between infectious and non-infectious enteric viruses remains a barrier to it practical use. Intercalating dyes can selectively penetrate the membranes of dead bacterial cells, bind to DNA, and prevent its amplification by PCR. It is possible that a similar method could be adapted for viruses.

Purpose: To develop a molecular-based method to distinguish between infectious and non-infectious noroviruses (NoV).

Methods: Murine NoV (MNV-1), a commonly used cultivable surrogate, and Snow Mountain virus (SMV, human NoV genogroup II.2) were heat treated to result in varying degrees of virus inactivation. Heated and unheated (control) aliquots were supplemented with 100 µM propidium monoazide (PMA), incubated in the dark for 30 min and then exposed to visible light for 5 min. RNA was extracted using the Trizol reagent, followed by RT-qPCR targeting the orf1 or the orf1/orf2 junction for MNV-1 and SMV, respectively. MNV-1 was also quantified by plaque assay on RAW 264.7 cells.

Results: When MNV-1 was heated at 65°C for up to 5 min and quantified by plaque assay and RT-qPCR with and without PMA pre-treatment, infectivity assay results correlated well with PMA-RT-qPCR; RT-qPCR without PMA pre-treatment over-estimated infectivity by about 3 $\log_{10}$. For SMV heated at 82°C for up to 15 min and quantified by RT-qPCR, an initial 3 $\log_{10}$ drop in virus titer was observed for the PMA-treated samples compared to no loss in virus titer for the non-PMA treated control sample. Results were more robust when the assay was applied to monodispersed virus suspensions, suggesting the method is sensitive to virus aggregation.

Significance: Nucleic acid intercalating agents show promise for use in distinguishing between infectious and inactivated NoV. With additional optimization, this may constitute a viable molecular approach to discriminate human NoV infectivity status in the absence of in vitro cultivation methods.

P2-100 Development of a Rapid Method for the Isolation and Detection of Hepatitis A Virus from Various Produce Items

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Introduction: Foodborne outbreaks attributed to human enteric viruses account for at least one third of all reported outbreaks in the United States. Hepatitis A virus (HAV), one of the most epidemiologically important of these viruses, has been continually implicated in several produce associated outbreaks. Due to the low levels of naturally occurring viral contamination and the lack of effective culture methods, detection of HAV from foods remains a challenge.

Purpose: The purpose of this research was to develop a rapid methodology for detecting low levels of HAV in spinach and tomatoes.

Methods: Twenty-five grams of baby spinach and cut tomatoes were surface or internally inoculated, respectively, with 10^2-10^6 PFU of the cytopathic HAV strain HM-175. A high concentration (4M) of a guanidine-based lysis buffer was used as a general denaturant to release viral RNA, which was subsequently precipitated with isopropanol and purified by a commercial RNA kit. Recovery efficiency was assessed by qRT-PCR. For tomatoes, virus recovery was also compared to a method utilizing an additional elution step and an enzymatic digestion step with 300 µl each of cellulase and pectinase.

Results: Consistent detection of HAV genome from both spinach and tomatoes was achieved at an inoculation level of 120 PFU per gram of food. Initial work identified that polyvinylpyrrolidone and potassium acetate reduced the carryover of polysaccharides and phenolics. When HAV was isolated from tomato slices, an initial elution step of 30 min at room temperature was as effective as enzymatic digestion. The detection limit per food sample was determined to be at least 50 PFU per PCR reaction.

Significance: A new, rapid (4–5 h) method was characterized to successfully detect low levels of HAV contamination from two very different food matrices, spinach and tomatoes. This method has the capacity to be further applied to other produce commodities.

P2-101 Effect of Amino Acids and Surfactants on RT-PCR Recovery of Viruses from Green Onions

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Introduction: Virus detection from fresh produce is often accomplished by elution into a buffer solution, with or without various additives, followed by PCR. Little information is available on the effect of these additives on virus recovery from produce or their effect on assay inhibition.

Purpose: The purpose of this study is to determine the effect of amino acids and surfactants on the recovery of MS2 bacteriophage, as an enteric virus surrogate, from surface-inoculated green onions.

Methods: Green onion in 4" pieces (10 g/sample) were surface inoculated with 10^5 pfu MS2 bacteriophage and air-dried in a biosafety cabinet. Samples were eluted by orbital shaking for 20 min at room temperature with 100 mM Tris buffer (pH 7.2) containing 0.5 M alanine, arginine, glycine, lysine, or threonine for amino acid trials; 5 mM SDS, 0.5% Tween 80 or 0.5% Triton X-100 for surfactant trials. RNA was extracted from all samples and quantitated via a real-time RT-PCR assay for MS2 bacteriophage along with a non-competitive internal amplification control (IAC).

Results: Based on the initial inoculum, percent recoveries from amino acid elutions were lysine (38.5 ± 25.0), arginine (37.4 ± 21.4), Tris (22.1 ± 9.8), threonine (20.7 ± 7.25), glycine (18.2 ± 10.9), and alanine (14.6 ± 4.7). Surfactant elution recoveries were SDS (47.6 ± 17.5), Triton X-100 (37.5 ± 28.7) Tween 80 (27.4 ± 26.2) and Tris (24.5 ± 12.2). Preliminary trials with hepatitis A virus inoculation also indicate that arginine/lysine, and SDS allowed the highest recoveries for amino acids and surfactants, respectively. None of the eluents showed more than 1 cycle difference from the no-matrix control samples, indicating that these additives do not cause reaction inhibition.

Significance: This study indicates that addition of certain amino acids and/or surfactants may allow enhanced recovery of viruses from contaminated green onions without causing inhibition of detection by PCR.

P2-102 Development of a Method to Concentrate Viruses from Fresh Produce Wash Water

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Introduction: Human norovirus is a common source of foodborne illness worldwide. Its low infectious dose requires concentration methods for detection from foods, but cannot be cultured in the laboratory. Murine norovirus (MNV-1) is frequently used as a human norovirus surrogate and can be enumerated using plaque assay.

Purpose: Development of an optimized method to concentrate MNV-1 from fresh produce wash water.

Methods: A cationic-charged magnetic bead separation technique (MBS; PATHATRIX) was compared to a centrifugal membrane concentration technique (CMC; Amicon) to concentrate MNV-1 from various wash waters (phosphate-buffered saline (PBS), deionized water alone and with organic material, and from spent water used to wash inoculated lettuce leaves. Wash water was inoculated to achieve concentrations between 2-log and 6-log PFU/mL. For MBS, 50mL inoculated water was incubated with the beads for 1 to 24 h at various temperatures. After processing, infectious viruses were quantified by plaque assay while bound to beads or after elution from beads by lowering the pH. The CMC method was performed by passing 15mL of inoculated wash water through a 30,000 molecular weight cut-off membrane at 2600*xg*. The wash water was concentrated with the unit either with or without a pre-filtering step to remove the organics.

Results: MBS yielded concentration factors of < 0.1 from the beads and between 0.5 and 1.4 from spent buffer in all samples tested, indicating viruses did not bind to the cationic-charged beads despite incubating samples with beads for a range of times and temperatures. CMC was effective at concentrating MNV-1, yielding an average concentration factor of 17.4 from all wash waters tested. CMC results remained consistent despite changing variables including filtered and unfiltered organics, recovery from lettuce or water, and starting virus concentration.

Significance: The use of antibody-bound beads or beads with a greater cationic charge should be investigated in the future to improve binding efficiency in the MBS method. Virus concentration using the CMC method is an effective method for concentrating low numbers of infectious virus from fresh produce wash water.

P2-103 The Impact of Four Buffer Systems Derived from a Matrix-solubilization Based Sample Preparation Protocol for Bacteria on the Integrity of Virus Particles

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Introduction: Major causes of foodborne illnesses in humans are viruses. Accurate detection of foodborne viruses is limited on molecular diagnostics, and existing methods are laborious, time consuming and not standardized. One important demand is the separation of the target particles from the food matrix as obtained by sample preparation protocols. Nevertheless, few investigations and protocols covering this topic are published so far. On the other hand, there are several newly developed and well established protocols for molecular biological quantification for foodborne bacteria available. The aim of this study was the investigation of the applicability of a recently developed sample preparation method that involves the solubilization of the food matrix (Matrix Lysis) for the separation of viruses from food samples.

Purpose: Testing of the impact of several food solubilization buffers as used for Matrix Lysis on the integrity of calicivirus and MS2 bacteriophage particles.

Methods: The influence of the four existing matrix lysis buffers on feline caliciviruses and MS2 phages was tested using reverse transcription RT-PCR for quantification. Feline caliciviruses and MS2 phages were used as models for norovirus. The particle count before inoculation was compared with the RT-PCR data after incubation in four buffer systems (A: 8M Urea and 1% SDS; B: 8M Urea and 1% Lutensol; C: 1 M $MgCl_2$; D: 7.5% Ionic Liquid: [emim]SCN).

Results: The recovery as obtained for the MS2 and calicivirus particles are in good agreement with the respective values as obtained for bacterial cells. Recovery rates are ranging from 31 to 70% in Buffers A and B and from 63 to 86% in Buffers C and D.

Significance: The Matrix Lysis system is a promising tool to support molecular biological detection of food-borne viruses. Future work will focus on concentration of viruses subsequent to Matrix Lysis and before RT-PCR detection.

P2-104 Chromogenic Indicators for Pathogens Contaminating Food Samples

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Introduction: Commercial indicators commonly evaluate food spoilage by measuring changes in temperature or pH-value but are not directly dependent on microbial growth. In the present study the applicability of chromogenic bacteria as an `on view` model is shown. Selected chromogenic bacteria placed on filter membranes and exhibiting similar growth characteristics as food pathogens mimic the development of possible pathogen flora. Resulting, a model comprising colored bacteria indicates microbial growth on food stuff.

Purpose: Development of a membrane-based approach for a sensitive model to facilitate *in situ* detection of possible food pathogen growth.

Methods: To proof osmotic nutrient supply for microbial growth but prevent contamination of the food with microbes, semi-permeable filter membranes with pore sizes ranging from 0.1 to 10 µm were used on plate and directly on minced meat or poultry. Controls were plated without filter membranes. As a model organism *Serratia marcescens*, producing the purple-red pigment prodigiosin, was used to test pore size related nutrient flow (colony sizes and numbers), biological integrity (growth on agar without nutrients) and impermeability (growth underneath the filter membrane). Growth was monitored over a period of 48 hours at various temperatures (4°C, 15°C, 25°C, 30°C). To test for minimal sensitivity, total microbial counts were determined for all meat samples (ISO/FDIS 4833).

Results: Similar colony numbers and diameters were detected comparing cell suspensions on filters and controls for pore sizes as far as 0.1 µm, indicating a good nutrient diffusion through the membranes. No colony growth was found upon removal of the filter membranes (0.1–0.4µm) and on sole agar plates indicating impermeability and biological integrity. Highly concentrated cell suspensions performed well on minced meat and poultry, developing red cell clusters on the filter membranes within four hours at 30°C. When signal occurrence was compared to total microbial cell counts from meat, a 0.4-log scale change of total microorganisms was detected.

Significance: The study presents a model for a food pathogen growth indicator, which can be adapted to various pathogenic organisms and food stuffs.

P2-105 Ionic Liquids for DNA Quantification Out of Gram Negative and Gram Positive Bacteria

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Introduction: In molecular biological food pathogen detection, an easy and quick method for bacterial cell lysis and DNA purification is of great interest. Time saving is a major reason for the application of molecular methods. In analytical chains including sample preparation the DNA isolation procedure is the most promising step for significant reduction of expenditure of time. Gram positive bacteria especially need extensive protocols, including over night enzymatic digestion to obtain DNA that can be used in sensitive analysis methods like qPCR.

Purpose: Establishing a fast and easy method for bacterial cell lysis and DNA isolation by using a two-phase system using a bmpyrr based ionic liquid (IL) and ddH_2O. Screening of ILs for DNA isolation out of Gram negative (*S.* Typhimurium, *E. coli*) and Gram positive (*L. monocytogenes*) bacteria. Inhibition of the following qPCR should be excluded.

Methods: As a reference method for quantitative DNA isolation, the NucleoSpin® tissue kit was used. The quantification of bacterial cell equivalents was carried out by qPCR with the respective protocols for *S.* Typhimurium, *E. coli* and *L. monocytogenes*.

Results: A ten minute protocol for cell lysis and DNA isolation out of various bacterial species was developed. DNA of *Salmonella* and *Escherichia* was found in high amounts. *Listeria* was isolated with an average yield of only 10% in comparison to the NucleoSpin® tissue kit. A pre-incubation step by using a DMAE-based IL was introduced for *Listeria* and resulted in a yield of about 100%. QPCR quantification demonstrated inhibition free amplification after the new DNA isolation protocol.

Significance: The two phase IL cell lysis protocol is able to lyse Gram negative cells within a few minutes and the DNA can be used directly afterwards for qPCR quantification. For Gram positive bacteria a pre-incubation step of ten minutes is necessary to obtain DNA recovery comparable to Gram negative bacteria.

P2-106 Evaluation of IBISA®, a New Selective and Chromogenic Method for the Detection of *Salmonella* Species in Foods

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Introduction: The use of chromogenic media for *Salmonella*'s detection in food samples has been described in different commercial methods. Nevertheless, most of these media, based on the detection of C8-esterase activity only, were reported to miss some strains such as *S.* Dublin or *S.* Bongori which express a weak enzymatic activity.

Purpose: The objective of this study was to evaluate the ability of the IBISA® Method to detect *Salmonella* in naturally or artificially contaminated foods and in pure culture against NF EN ISO 6579 standard using XLD and the conventional C8-esterase-based chromogenic agar plate as screening media.

Methods: Food samples were 1:10 diluted in Buffered Peptone Water, then supplemented with ISS (IBISA® Specific Supplement) and incubated 16-20 h at 41.5°C. Ten µl of the enriched broth were streaked on IBISA® plate and incubated 24 +/-3 h at 37°C. On this new medium, *Salmonella* grow as typical large green colonies due to a specific mix of chromogenic esterase substrates. An inclusivity study was performed with 90 strains of *Salmonella* including lactose-positive strains, *S.* Dublin and non-motile *Salmonella*. The exclusivity study was carried out on 35 non-*Salmonella* strains. 174 food samples including naturally contaminated samples were tested according to the NF EN ISO6579 reference method and IBISA® Method. Relative Accuracy, sensitivity and specificity were calculated during this evaluation.

Results: The inclusivity and exclusivity study have shown a perfect ability of the IBISA® to detect all *Salmonella* and to discriminate non-*Salmonella* strains. In the meantime, XLD or classical chromogenic media demonstrated some difficulties to recover atypical strains such as *S.* Seftenberg and *S.* Dublin. On the 174 food samples, 55 were found positive and 116 were negative with both IBISA® and NF EN ISO 6579 methods. 3 additional positive samples were detected and confirmed positive only with IBISA® method.

Significance: In these trials, 48 hours IBISA® protocol showed comparable and even better sensitivity in comparison to the reference method for *Salmonella* detection in a variety of foods. Moreover the use of an esterase substrates mix allows IBISA® to detect *Salmonella* strains which are usually difficult to recover on classical chromogenic media.

P2-107 A Comparative Evaluation of the ChromID™ Ottaviani Agosti Agar (OAA) for the Detection and Enumeration of *Listeria monocytogenes* and *Listeria* Species

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Introduction: ChromID™ Ottaviani Agosti (OAA) agar is a chromogenic medium for the selective isolation, enumeration and presumptive identification of *L. monocytogenes* and *Listeria* species in food and agri-food production environmental specimens. The agar contains a nutritive base combining different peptones and two substrates, including a chromogenic one. It favors growth of all *Listeria* spp.and reveals the presence of certain enzyme activities. *Listeria* grow on the agar and produce blue-turquoise colonies (glucosidase activity). The differentiation of *Listeria monocytogenes* is based on the appearance of an opaque halo around the colony (phospholipase C activity). The formulation inhibits the growth of most other bacteria and yeasts.

Purpose: This new method was independently validated for the detection and enumeration of *L. monocytogenes* and *Listeria* species as part of the AOAC-RI PTM validation process.

Methods: The OAA method was compared to the USDA FSIS MLG 8.06 reference method for chicken franks and stainless steel environmental samples and FDA BAM for raw shrimp. For the detection portion, 20 replicates were analyzed at one inoculum level: 0.2-2 CFU/25 g for chicken franks, 0.2-2 CFU/5 sq cm for the stainless steel, and 2-20 CFU/25 g for the raw shrimp. Five controls were analyzed at 0 CFU/25 g. For the enumeration portion, 15 samples (3 lots of 5 samples) of chicken franks and raw shrimp were analyzed at 3 levels: 100 CFU/g, 1,000 CFU/g and 10,000 CFU/g.

Results: The OAA and USDA method results (chicken franks, stainless steel) were comparable for detection of *Listeria* spp. $\chi 2$ values = 1.26 for chicken franks and 0.10 for stainless steel, indicating no significant difference. For raw shrimp, the OAA and FDA BAM results were comparable for detection of *L. mono* with a $\chi 2$ of 0.00. For enumeration, the OAA and USDA results for chicken franks & OAA and BAM results for raw shrimp were comparable. The *P*-values for chicken franks were 0.437, 0.050 and 0.008 for the low, medium and high levels, respectively. *P*-values for the raw shrimp were 0.134, 0.196 and 0.005 for the low, medium and high levels, respectively. There was a significant difference in test and reference method results at the high level for chicken franks and shrimp. For both matrices, the OAA method had a better repeatability value than the USDA or FDA reference methods.

Significance: This new method is a reliable alternative to the traditional selective agars for detection and enumeration of *L. mono* and *Listeria* species in food and environmental samples.

P2-108 Performances Assessment of the ChromID™ L. mono agar Method According to the ISO 16140 Standard for *Listeria monocytogenes* Enumeration in Food and Environmental Samples

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Introduction: The chromID L. mono method is a new *Listeria monocytogenes* enumeration method based on a chromogenic selective agar. The users can choose the plating protocol (pour or surface), as well as the confirmatory tests they want to realize in their routine analyses.

Purpose: A study was conducted at ADRIA, to validate this new method in comparison to the ISO 11290-2 reference method, as part of the Microval approval process and according to the ISO 16140 standard.

Methods: Samples diluted in BPW are inoculated onto chromID L.mono plates. The reading of the plates can be done after one day incubation time. Flexibility on incubation time is available, as well as storing the plates for 3 days at 4°C before enumeration. During the validation study,

all these protocols were tested, as well as the proposed confirmatory tests: (i) the ISO 11290-2 tests on one purified colony, by (ii) a RAPIDEC or API gallery on one isolated colony without purification step, (iii) the VIDAS LMO2 or Accuprobe LMO on one colony, isolated or not, without purification step.

Results: Whatever the plating technique, the incubation and storage times, as well as the confirmatory tests, the chromID L. mono method showed satisfactory relative linearity performances, with linear correlation coefficients higher than 0.98. Biases between both methods were characterized by low values, varying from -0.215 to + 0.025 log CFU/g. The intercepts close to 0 and the slopes close to 1 were validated for all the tested categories in the accuracy study. Selectivity and specificity of the method were shown to be good by testing 30 target and 22 non-target strains. The limits of repeatability and reproducibility of the chromID L. mono method, which were calculated during an inter-laboratory study involving 13 labs, were similar to those of the 11290-2 standard method. The biases between the compared methods were clearly non significant, varying from -0.00 to -0.06 log CFU/g.

Significance: According to these performances assessments, the chromID L. mono agar method represents a valuable and user-friendly alternative method for *L. monocytogenes* enumeration in food and environmental samples. Final results are obtained the next day by using a Rapidec gallery, the VIDAS LMO2 assay or the Accuprobe L. mono assay as confirmatory tests.

P2-109 A New Immuno-concentration Assay for Shiga-like Toxin *Escherichia coli,* Used as Sample Preparation before Real-time PCR for Virulence Genes Detection

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Introduction: The high virulence status of Shiga toxin-producing *Escherichia coli* (STEC) means that absence of these pathogenic *E. coli* strains must be guaranteed to ensure a safe release of foods.

Purpose: VIDAS UP *E. coli* serogroups (ESPT) was developed with the use of 5 phage recombinant proteins for the immuno-concentration (IC) of *E. coli* serogroup O26, O103, O111, O145 and O157 from food enrichments. Used before PCR assays for virulence genes, the new test is designed to reduce the number of non-relevant positive reactions and facilitates further confirmation of pathogenic strains. This study was designed to demonstrate the feasibility of the immuno-concentration assay and to evaluate its sensitivity.

Methods: The limit of detection was determined by spiking between 10^1 and 10^7 CFU/ml of each of the five serogroups into ground beef enriched buffered peptone water (BPW) and detecting PCR signal. A food study was conducted on 88 naturally contaminated products enriched in BPW for 16-20 hours at 41.5°C. Enrichments were tested for the presence of *stx* and *eae* genes with RT-PCR assays performed directly on the enrichments or after the immuno-concentration assay. The immuno-concentrate was also used for plating on a selective agar to confirm the presence of a pathogenic strain as an alternative to IMS.

Results: The inclusivity and exclusivity study clearly showed that the assay was highly specific for detection of the targeted strains. The limit of detection of O26, O103, O111, O145 and O157 cultures was found to be at least 10^4 cells/mL. The test was shown to be sensitive enough for isolation of low levels of stressed strains (1 to 5 CFU/25 g) in artificially contaminated foods such as raw meat. The IC assays eliminated 75% of non STEC PCR positive results from 88 suspected positive samples. Compared to traditional IMS for confirmation, the new test showed 88% concordance. One *E. coli* O103 strain was not recovered after IC and an additional O103 strain was recovered only after the IC assay. Less background flora was present on the plates and the recovery of serogroups of interest was greatly facilitated.

Significance: This study has demonstrated that the VIDAS ESPT assay is a promising tool to isolate *E. coli* O157, O26, O103, O111 and O145 from food enrichment, prior to establishing their pathogenic status with a PCR assay. It significantly reduced the number of non-relevant PCR positive results and so the number of confirmation. This automated method will provide technical and economic advantages to the food industry for routine testing by reducing the number of false positive PCR screen samples that need to be run through the confirmation process.

P2-110 ISO 16140 Validation of a New Agar Plate Method for the Detection of *Campylobacter*

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Introduction: To release food products at the production stage, international rules require to use a reference method or an alternative method certified by a third party organization such as AFNOR, Microval or AOAC.

Purpose: The goal of this study was the validation of the CampyFood method for detection of *Campylobacter* in food products and environmental samples, according to ISO 16140 guidelines.

Methods: Food samples, 1:10 diluted in a new ready-to-use proprietary selective broth without horse blood, the CampyFood broth, were enriched for 48 hours at 41.5°C. A microaerobic atmosphere was generated directly into a specific stomacher bag, by addition of a gas generator in a small pocket of the bag. After incubation, 10 µl of enrichment broth were streaked onto CampyFood agar and the plates were incubated for 40–48 h at 41.5 °C in a microaerobic atmosphere. Presumptive *Campylobacter* red colonies were confirmed by the VIDAS *Campylobacter* assay or by traditional assays. The preliminary study and the interlaboratory study were conducted as part of the AFNOR Certification approval process and according to ISO 16140 standard.

Results: For the comparative study, 64 poultry products, 82 meat products and 62 environmental samples were analyzed. Using the Mac Nemar's analysis at the 5% level, the methods were shown to be not comparable but the alternative method gave significantly better results with 97 samples found positive compared to 85 for the reference method. Good results were obtained in the inclusivity and exclusivity studies with 50 *Campylobacter* and 30 non *Campylobacter* strains. The 50% detection limit was found to be between 0.2 and 1.8 CFU/25 g for the new method and between 0.2 and 2.4 CFU/25 g for the reference method. The interlaboratory study did not show comparable values of relative accuracy, specificity and sensitivity for the alternative method and the reference method but performance of the new method was significantly better

Significance: The CampyFood agar method was certified as an alternative method to the reference ISO 10272-1 reference method for the detection of *Campylobacter* in poultry, meat and environmental samples. This study demonstrated that the alternative method was much better than the reference method for the recovery of thermotolerant *Campylobacter*, in samples containing high background flora. The ready to use broth and the specific bag to promote microaerobic atmosphere greatly simplify and enhance the practicability and performances of the method.

P2-111 Strains of the Major Non-O157 Shiga Toxin-producing *Escherichia coli* Serogroups Exhibit Growth Patterns Similar to O157:H7 at 10 °C in Lean Ground Beef

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Introduction: Concerns have arisen about the potential public health burden attributable to non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains in the United States meat supply.

Purpose: The aim of this study was to compare the growth of six clinical-isolate non-O157 STEC strains from serogroups O26, O45, O103, O111, O121, O145 with one O157:H7 STEC strain in lean ground beef (7% fat) at 10°C.

Methods: Cultures were prepared by incubating in brain-heart infusion broth (BHIB) at 37°C for 24 h and diluting in BHIB to ~10^5 $CFUml^{-1}$. Prepared cultures (1ml) were used to inoculate bags containing 25 g meat (~10^4 $CFUg^{-1}$) which were then stored at 10°C for up to 72 h (3 trials/strain). Inoculum level was comparable to the level of native microflora of the raw ground beef as measured by APC. At 0, 12, 15, 18, 24, 36, 48, and 72h, one bag of inoculated meat was stomached for 30 s with 99 ml Butterfield's Phosphate Diluent (BPD) and serially diluted in BPD. Inoculum cells were enumerated on modified Eosin Methylene Blue agar with bile salts (0.15%) added to inhibit indigenous microflora. The no-asymptote Baranyi model was fitted to data using DMFit, and mean lag phase duration (LPD) and mean maximum growth rates (μ) were generated.

Results: Strain *E. coli* O26:H11 survived but did not grow. For other strains, LPD ranged from 11.3h to 39.4 h, and μ ranged from 0.032 $\log_{10}CFUg^{-1}h^{-1}$ to 0.045 $\log_{10}CFUg^{-1}h^{-1}$. Strains in serogroups O145 and O157 had significantly shorter LPD than strains in serogroups O45, O103, and O121 ($P < 0.05$), while strain O111 had an intermediate LPD. Significant inter-strain differences related to LPD values occurred at 24 h and later ($P < 0.05$). After lag-phase ended, there were no significant differences in μ ($P \geq 0.05$).

Significance: Results suggest that strains of O157 and non-O157 STEC serogroups can grow under inadequate refrigeration temperatures. While additional work is required to determine the behavior of other strains within these serogroups, this study supports increased regulatory concern surrounding non-O157 STEC in meat.

P2-112 Strains of the Major Shiga Toxin-producing *Escherichia coli* Serogroups Exhibit Similar Thermal Tolerance in Lean Ground Beef

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Introduction: Escherichia coli O157:H7 is an adulterant in raw non-intact beef and its presence therein leads to a Class I recall. Regulators in the United States are considering the designation of six serogroups of non-O157 Shiga toxin-producing *E. coli* (STEC) as adulterants: O26, O45, O103, O111, O121 and O145.

Purpose: We compared the thermotolerance of representative strains from each of the six STEC serogroups with reference O157:H7 strains at 65.5°C in lean ground beef (7% fat). The reference strains included four beef-trim isolates, selected based on preliminary work indicating high heat tolerance, and an outbreak-linked strain (ATCC 43895).

Methods: Small Whirl-Pak bags containing 25 g preheated ground beef (65.5°C) were inoculated with 1 ml aliquots of stationary-phase culture (~10^9 CFU/ml), and immediately transferred to a shaking water bath (65.5°C). At each sampling time (0–120 s), one bag of meat was removed and held on ice for 10 min to reach ≤ 4°C. The meat was everted into a stomacher bag and stomached with 99 ml Butterfield's Phosphate Diluent (BPD) for 30 s. Serial dilutions were made in BPD and viable cells enumerated by plating on modified Eosin Methylene Blue agar (37°C for 24 h). $D_{65.5°C}$-values were calculated from $\log_{10}$CFU/g data, with at least 3 trials per strain, and compared using analysis of variance.

Results: $D_{65.5°C}$-values ranged from 1.16 min (ATCC 43895) to 0.76 min (O26:H11). None of the strains exhibited thermotolerance greater than reference strain ATCC 43985. *E. coli* O111:H8, 0103:H2 and 026:H11 exhibited significantly lower thermal tolerance than ATCC 43895 ($P < 0.05$), while none of the non-O157 strains exhibited thermal tolerance different from the four beef-trim isolates ($P \geq 0.05$).

Significance: These results suggest that thermal-processing intervention treatments that target destruction of *E. coli* O157:H7 may have adequate lethality against other STEC. However, further studies should be conducted with other strains in each of the six major non-O157 serogroups.

P2-113 *Salmonella* and *Escherichia coli* O157:H7 Prevalence on Beef Carcasses in a Non-TIF Harvest Plant in the Mexican Yucatan

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Introduction: Non-TIF (Federal Inspection Type) regulated beef processing plants in Mexico are a major concern for this country and especially its young population, because foodborne illness is the number one cause of death for children ages 1-4. Non-TIF plants provide product to central markets and to vulnerable populations.

Purpose: To determine the prevalence of *Salmonella* and *E. coli* O157:H7 on beef carcasses from a Non-TIF processing plant in Merida, Mexico.

Methods: The prevalence of *Salmonella* and *E. coli* O157:H7 on carcasses during three areas of the slaughter process was determined. A total of 90 swab samples (30 hide, 30 pre-evisceration and 30 post-evisceration) were collected in each of two trips to Mexico. Sterile sponges pre-hydrated with buffered peptone water were used to collect a composite sample from the fore shank and midline area of each animal. Samples were transported to Texas Tech University under cold conditions for microbiological analysis within 2 days after collection. *Salmonella* and *E. coli* O157:H7 presence were confirmed using the Dupont Qualicon BAX® system.

Results: No *E. coli* O157:H7 was detected in any of the samples. Overall, the *Salmonella* prevalence was 100% positive for all hide samples taken on both occasions. At pre-evisceration, 93% of the carcasses were positive for *Salmonella* in September and 100% positive for *Salmonella* in December. The percentage of positive *Salmonella* samples post-evisceration was 93% and 46% in September and December, respectively.

Significance: Determining the baseline in non-TIF regulated processing plants will contribute to the understanding and to pinpoint locations inside the plant where improvements are needed in food sanitation, training and dressing procedures, and where to implement interventions thus, helping to improve the quality and safety of meat products sold to Mexican consumers.

P2-114 Prevalence of Toxin-containing Non-O157 *Escherichia coli* Found in Commercial Ground Beef

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is a pathogenic subset of *E. coli*. It can cause serious disease and complications such as hemolytic uremic syndrome (HUS) in an infected person due to toxins present in these bacteria. The most frequent serotype associated with EHEC is O157, but others are emerging as the cause of outbreaks and HUS. Commonly infection occurs from eating contaminated food, such as ground beef.

Purpose: To investigate the prevalence of EHEC contamination, in particular the non-O157 serotypes of *E. coli*, in commercially available ground beef and identify the toxin genes present in the bacteria that are associated with the EHEC profile.

Methods: Packaged ground beef was purchased from several chain grocery stores in ten states. Samples from each, totaling 5070, were enriched and then cultured on MacConkey agar to determine the presence of *E. coli*, and used in PCR for detection of the specific toxin genes stx_1 or stx_2, and *eae* or *subAB*. A separate PCR was performed on the positive samples to determine the serotype.

Results: There were 104 (2.0%) EHEC-positive, O157-negative isolates from 5070 ground beef samples. Thirty (0.6% of the total ground beef samples) were of the CDC-6 serotype group, including O26, O103, O111, O121 and O145. but not O45. In this group of *stx*-positive isolates, 26 of the 30 isolates contained *eae* and 5 contained *subAB*. Another 14 (0.28%) *E. coli* isolates were of the O113 serotype and all of these contained only stx_2 and *subAB*.

Significance: Currently O157 is the only *E. coli* declared as an adulterant by USDA for food recall, hence standard testing is for this serotype. However, given the occurrence of other serotypes in outbreaks and in food, including these as a part of the testing methods should help to ensure the safety of the food supply.

P2-115 *Escherichia coli* O157:H7 Strains Differ in Their Phenotypic Responses as Measured Using Phenotype Microarrays

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Introduction: Comparative genetic studies have provided some insights into the genetic differences between strains of the foodborne pathogen *E. coli* O157:H7, which can vary in their ability to cause severe human disease. Most strains of *E. coli* O157:H7 have a high level of sequence identity in their genomes so comparative genomics provides limited insight on virulence potential. Differential analysis of a broad range of phenotypic characteristics between *E. coli* O157:H7 strains would provide a means of directly measuring characteristics related to pathogenicity as well as providing direction for comparative genomic analysis. Phenotype Microarrays™ (PM) were developed to enable high throughput analysis of large numbers of phenotypic properties.

Purpose: The purpose of this study was to compare the phenotypic differences between Australian cattle strains of *E. coli* O157 and an outbreak strain using PM.

Methods: Five *E. coli* O157:H7 strains (four from Australian cattle and the Sakai outbreak strain from Japan) were tested using PM to screen 1,920 different tests for metabolic and chemical sensitivities. The PM results from each of the Australian strains were reported as phenotypes lost or gained compared to *E. coli* O157:H7 Sakai.

Results: The four Australian strains produced fewer positive phenotypic tests in the PM than Sakai, with the Australian strains gaining between 1 to 18 phenotypes over Sakai, but losing between 17 to 96 phenotypes depending on the individual strain. The most pronounced differences were loss of phenotypes in the Australian strains associated with chemical sensitivities. In particular, Australian strains appeared to be more sensitive to folate antagonists such as sulfamethoxazole, sulfathiazole and sulfisoxazole.

Significance: The increased sensitivity of these Australian *E. coli* O157:H7 strains to various chemicals requires further investigation to determine if such phenotypes play a role in decreased survival and transmission to humans and potentially lower virulence.

P2-116 Effect of Sanitizer Stress on the Growth Kinetics of Various Pathogenic *Escherichia coli* in Broth

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Introduction: Escherichia coli in fresh produce and equipment encounter mild stress from various sanitizers during processing. Resistance to these stresses and ability to repair their injuries during storage may affect the safety of human health. Although many studies of stress responses in non-pathogenic *E. coli* and *E. coli* O157:H7 have been performed, differences in response from sanitizer stress among pathogenic *E. coli* strains have not been compared.

Purpose: The objective of this study was to compare growth kinetics of two pathogenic *E. coli* strains sub-lethally stressed due to the sanitizer in broth stored at 10, 13, 15, 24 and 30°C.

Methods: Pathogenic *E. coli* (ATCC 25922) and *E. coli* O157: H7(NCTC 12079) strains in stationary phase were stressed for 5 min at room temperature with 75 or 100 ppm sodium hypochlorite. The sanitizer was withdrawn and stressed cultures were diluted in sterilized tryptic soy broth for neutralization and to reach appropriate initial population of 2.5-3.5 log CFU/ml. Broth containing stressed pathogenic *E. coli* and *E. coli* O157: H7 were incubated at 10, 13, 15, 24 and 30°C without agitation, and lag time (LT) and specific growth rate (SGR) of each growth curve were compared.

Results: There were differences in minimum growth temperature between pathogenic *E. coli* and *E. coli* O157:H7. Unstressed *E. coli* O157:H7 and pathogenic *E. coli* survived at 10°C and 15°C, respectively. Stressed *E. coli* O157:H7 was able to grow at 13°C, while stressed pathogenic *E. coli* was not able to growth at even 15°C. LT and SGR were mainly affected by the extent of stress at each temperature.

Significance: 100 ppm Sodium hypochlorite has been used for fresh produce in foodservice. The growth kinetics of pathogenic *E. coli* varied with the strength of sanitizer, storage temperature and strain. Therefore, the potential risk of survival of stressed pathogenic *E. coli* in washed fresh produce with sanitizer must be carefully considered.

P2-117 Characterization of Cell Surface Properties, Chlorine Resistance and Attachment of *Escherichia coli* O157:H7 Strains

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Introduction: Escherichia coli O157:H7 outbreaks linked to tainted fresh produce have increased during recent years. It is important to understand better how this pathogen survives post-harvest processing so that more effective risk reduction measures can be implemented.

Purpose: The aim of this study is to characterize the cell surface properties, chlorine resistance and attachment to leafy greens of several *E. coli* O157:H7 strains belonging to different phylogenetic clades, in order to determine if there is any correlation between their survival and bacterial cell surface properties.

Methods: Six strains chosen among the nine *E. coli* O157:H7 clades included the fresh produce outbreak associated strains Sakai (1996 sprout outbreak in Japan) and TW14359 (2006 spinach outbreak in the US). The growth kinetics of these strains were compared in BHI broth containing 0.81 ppm free chlorine. Cell surface hydrophobicity, auto-aggregation and curli production were determined by in vitro assays and compared to the generic *E. coli* strain BW25113.

Results: The results showed that these *E. coli* O157:H7 strains exhibited different chlorine resistances regardless of their phylogenetic distance. They were approximately 20% more hydrophilic ($P = 0.0072$) and showed 77% less auto-aggregated cells ($P = 0.049$) after overnight incubation in broth than the generic *E. coli* strain. However, no distinguishable hydrophobic or aggregation properties were observed among the six *E. coli* O157:H7 strains despite their different resistances toward chlorine treatment. Curli production measured by a Congo-Red binding assay in

suspension varied among the strains and exhibited no correlation with chlorine resistance. In preliminary studies of chlorine exposure (0.81 ppm) of cells being attached to spinach leaf surface, the spinach outbreak strain TW14359 showed less inactivation (2.6-fold) than the sprout outbreak strain Sakai (15-fold).

Significance: The information generated from this study will help to develop more effective interventions used in the produce industry.

P2-118 Genetic and Phenotypic Characterization of Shiga Toxin-producing *Escherichia coli* Isolates Originating from British Columbia, Canada

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are capable of producing severe human disease through foodborne transmission. Little data exist regarding the genetic diversity or antimicrobial resistance (AMR) associated with STEC strains causing disease in British Columbia (BC).

Purpose: The study aims to investigate the genetic diversity and AMR of STEC causing human disease in BC.

Methods: Twenty-five STEC strains submitted to the BC CDC were randomly selected for analysis. All strains were serotyped and AMR phenotypes determined using a panel of 16 antimicrobials and standard disc diffusion assays. Genotypically, PCR was used to detect virulence genes (*stx1*, *stx2*, *eaeA*, and *hlyA*), and plasmid and pulsed-field gel electrophoresis (PFGE) typing were performed. In strains possessing AMR, transformations and/or conjugations were used to determine location and mobility of AMR determinants. PCR and DNA sequencing were used to confirm the presence of bla_{CMY-2}.

Results: All STEC (10 O157:H7 and 15 non-O157:H7) were positive for *stx1* and/or *stx2* and 24 strains were positive for *eaeA* and *hlyA*. STEC were sensitive to most antimicrobials, with only three strains resistant to ≥4 antimicrobials. One *E. coli* O157:H7 strain resistant to ampicillin was also resistant to ampicillin/clavulanate, cefoxitin, and ceftazidime. PCR, DNA sequencing, and conjugation experiments confirmed the presence of bla_{CMY-2} on a large, conjugative plasmid (70 kb). AMR phenotypes in all STEC were transferable by electroporation, indicating plasmidic rather than chromosomal origin. Overall, 24 strains possessed plasmids ranging in size from 3.5-100 kb; one strain lacked plasmids. Despite no epidemiological link, two O121, O157, and O165 strains, respectively, possessed identical PFGE types, virulence and similar antibiogram profiles.

Significance: PFGE, plasmid, virulence and AMR typing revealed evidence of genetic diversity and clonality within the sampled population. Further work is required to examine selection pressures and possible dissemination routes of STEC within BC. Also, the presence of mobile bla_{CMY-2} and the plasmidic association of all AMR phenotypes demonstrates the mobile nature of STEC AMR in BC.

P2-119 Screening Non-O157 Shiga Toxin-producing *Escherichia coli* Serotypes on Agar Media by Hyperspectral Imaging

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a group of pathogenic bacteria causing serious human illnesses. Recently, many cases of infections have occurred due to non-0157 STEC serotypes, such as 0103, 0111, and 026. It is well known that samples may be screened for O157 serotypes by culturing on sorbitol-Mac-Conkey agar for 24 h. However, non-0157 serotypes ferment sorbitol and a fermentable carbohydrate scores for discrimination are not available.

Purpose: To investigate the potential of VNIR hyperspectral imaging and chemometrics to spectrally differentiate six most common non-O517 STEC serotypes (O26, O45, O103, O111, O121, and O145) on agar media.

Methods: Pure cultures of six spots were inoculated on rainbow agar plates in duplicate. The plates were incubated for 24 h. Each inoculated spot was 5µL with 10^8 cells/ml fluid suspension. The Themis Vision Systems' hyperspectral imaging system was used to acquire images from 400 nm to 900 nm. Regions of interest associated with colonies were created for developing a prediction model. PCA was used to visualize data clusters and to develop a prediction model. The prediction model using 6 PCA components was then applied to hyperspectral images for validation. The accuracy of the predicted PCA scores was evaluated by the Mahalanobis distance classifier.

Results: PCA score plots of the first derivatives revealed potential separability of the serotypes. The prediction with six PCA components showed an overall detection accuracy of 94%. Detection accuracy of O26, O45, and O111 was 99% with less than 2% false positive and negative errors. The detection accuracy of the other serotypes varied from 83% (O121), 86% (O103) to 95% (O145).

Significance: The potential of combining hyperspectral imaging and chemometrics to differentiate non-O157 STEC serotypes was demonstrated. Hyperspectral imaging can improve the speed and accuracy of the current presumptive screening practice routinely done in food and regulatory laboratories.

P2-120 Reducing Pathogenic *Vibrio* spp. in Oysters Using a Thermal Post-harvest Process (PHP)

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Introduction: Pathogenic *Vibrio* spp. are the most common causes of foodborne disease associated with the consumption of raw or undercooked seafood, especially molluscan shellfish. There is a growing need for the implementation of post-harvest processes (PHP) to reduce *V. vulnificus* and *V. parahaemolyticus* to insignificant levels to prevent infection and meet FDA regulations.

Purpose: The purpose of this study was to modify an existing heat-shock treatment routinely used by North Carolina shellfish dealers to facilitate oyster shucking. The heat shock method was evaluated to determine its efficacy in reducing pathogenic *Vibrio* levels to meet FDA requirements for recognition under the Interstate Shellfish Sanitation Conference guidelines as an approved PHP method.

Methods: Under pilot-scale laboratory conditions (50 gal stainless steel tank with recirculating system and direct steam injection), freshly harvested summertime oysters were thermally treated at 60 ± 0.5°C for 2, 4, 6, and 8 min and quickly chilled to preserve sensory qualities. Control and treated oysters were quantitatively analyzed for surviving *V. vulnificus* and *V. parahaemolyticus* populations using MPN methods described in the FDA Bacteriological Analytical Manual.

Results: Log_{10} reductions for *V. vulnificus* at 60°C were 0.8 for 2 min, 2.4 for 4 min, 3.9 for 6 min, and 3.8 for 8 min. Log_{10} reductions at 60°C for *V. parahaemolyticus* were 0.4 for 2 min, 2.7 for 4 min, 4.2 for 6 min, and 4.5 for 8 min. A treatment of 6-8 min at 60°C was necessary to reduce *V. vulniificus* and *V. parahaemolyticus* by about 4 log_{10}, which approaches levels associated with significant reductions in public health risk.

Significance: This pilot-scale study demonstrates that heat-shock treatment to facilitate oyster shucking may also be effective in reducing pathogenic *Vibrio spp.* to non-detectable levels. Equipment and process validations are necessary to ensure meeting FDA requirements. Commercial upscale and accompanying sensory studies are currently underway.

P2-121 Ethanol Treatment to Inactivate Feline Calicivirus and Murine Norovirus as Norovirus Surrogates on Stainless Steel Surfaces

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Introduction: Viral outbreaks have been increasing every year throughout the world. In particular, norovirus (NoV) frequently threatens consumers and has generated many patient incidents in South Korea. Ethanol is a popular disinfectant to prevent cross-contamination of NoV on stainless steel as a food contact surface.

Purpose: This study investigated the efficacy of ethanol in removing feline calicivirus (FCV) and murine norovirus (MNV) as surrogates of NoV on stainless steel surfaces and provides predictive inactivation models.

Methods: 3D response surface methodology (RSM) was used for the predictive inactivation models. And the reduction levels of FCV and MNV on stainless steel surfaces after treatment of various concentrations (0-70%) of ethanol during a range of treatment times (0-10 mins) were investigated.

Results: The reduced amounts of FCV and MNV on stainless steel after treatment of 70% ethanol were 2.52 TCID50/coupon and 4.58 TCID50/coupon, respectively. The survivability of FCV was higher than that of MNV, and this study demonstrates that ethanol treatment has superior ability to reduce MNV than FCV. The polynomial equations predicting the inactivation of FCV and MNV were as follows: FCV (log TCID50/coupon) = +0.19379 + 0.067282x1 + 0.058945x2 - 8.57143E-004x1x2 - 1.44483E-003x12 - 3.51935E-004x22 (x1 ; time and x2 ; concentration); and MNV (log TCID50/coupon) = +1.08790 + 0.65635x1 + 0.077860x2 - 1.47143E-003x1x2 -0.024552x12 - 6.56158E-004 x22 (x1 ; time and x2 ; concentration). The predictive inactivation models by RSM fit well (R^2 = 0.9621 and 0.9088, respectively), and were expressed as adequate models by Prob > F-value ($P < 0.0001$).

Significance: The results indicate that FCV and MNV can be controlled on stainless steel surfaces through ethanol treatment and predictive inactivation models can be applied to control NoV on food contact surfaces in foodservice facilities and food manufacturing plants.

P2-122 Molecular Typing of *Vibrio parahaemolyticus* Isolates from the Middle East Coast of China

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Introduction: The outbreaks of *Vibrio parahaemolyticus* gastroenteritis in China highlight the need for strain characterization and differentiation of this pathogenic species. Molecular typing of *V. parahaemolyticus* was shown as a useful tool for tracking the source of infection and detection of virulent strains, as well as for determining the geographical and host distribution of possible variants.

Purpose: Since one typing method may not distinguish *V. parahaemolyticus* outbreak isolates that share a high level of genetic similarity, and the main objective of this study was to compare four existing methods for subtyping *V. parahaemolyticus* strains. In this study several methods were used to determine if we could develop a procedure that would provide satisfactory discrimination of pathogenic strains in a collection of 56 isolates collected along the middle-east coastline of China.

Methods: A total of 56 epidemiologically-unrelated strains of *V. parahaemolyticus* were isolated from clinical samples, seafood and various environmental sites in the middle-east coastline of China between 2006 and 2008. The isolates were characterized using four various molecular typing methods, including ribotyping, enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR), pulsed-field gel electrophoresis (PFGE), and sequence analysis of the *gyrB* gene.

Results: Genetic profiles of cluster analysis from these molecular typing tests clearly showed that there were differences in potential pathogenicity among isolates from seafood and its environments. Genetic characterization of two isolates (F13 and QS2) that originated from seafood demonstrated that they were potentially pathogenic. Discriminatory indices of four typing methods for the 56 *V. parahaemolyticus* isolates were differentiated by Simpson's Index of Diversity. The discriminatory index of ERIC-PCR typing was maximal (D was equal to 0.942), while that of sequence analysis of the *gyrB* gene was minimal (D was equal to 0.702). The discriminatory ability was greatly enhanced (D was equal to 0.966) when ERIC-PCR was coupled with sequence analysis of the *gyrB* gene. These results suggest that ERIC-PCR combined with sequence analysis may be a reliable, rapid typing strategy for *V. parahaemolyticus* strains.

Significance: Due to the large number of human disease outbreaks and the consequent economic loss for aquaculture resulting from *Vibrio parahaemolyticus*, correct identification, classification, and tracing of the transmission of this organism becomes an issue of great importance.

P2-123 Characteristics of *Vibrio* spp. Strains Isolated from Seafood at Retail in Switzerland

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Introduction: The genus *Vibrio* includes important human pathogens such as *V. cholerae*, *V. parahaemolyticus* or *V. vulnificus*. *Vibrio* spp. are widespread in coastal marine waters and some species, especially *V. parahaemolyticus*, are regularly linked to gastroenteritis caused by consumption of raw, undercooked or recontaminated seafood.

Purpose: The aim of this study was to assess the occurrence of *Vibrio* spp. in seafood at retail and to further characterize isolated strains.

Methods: Samples comprised 102 fish fillets, 34 bivalves, and two sepias. Isolation of *Vibrio* spp. was accomplished by culture (TCBS and chromID vibrio agar) after enrichment (ASPW) in accordance with ISO 21872-1/2:2007. Presumptive isolates were identified by API 20E and species-specific PCRs. *V. cholerae* strains were agglutinated with polyvalent O1 and O139 somatic antisera and tested by PCR for the cholera toxin subunit A gene (*ctxA*). *V. parahaemolyticus* strains were tested by PCR for genes encoding thermostable direct hemolysin (*tdh*) and TDH-related hemolysin (*trh*).

Results: Vibrio spp. were found in 32.6% of the 138 samples. *V. cholerae* was isolated from three (fillets, sepia) and *V. parahaemolyticus* from eight samples (mussels, fillet). None of these strains harbored species-specific virulence factors. Besides, *V. alginolyticus* was isolated from 40 samples (mussels, fillets, sepia) and *V. fluvialis* from one sample (fillet). With regard to seafood, the risk associated with *V. alginolyticus* is limited, whereas *V. fluvialis* has been reported as a cause of serious gastrointestinal disease. Thus, highly pathogenic strains seem to occur only at low numbers, but the use of solely cultural detection methods could be a limitation.

Significance: The results obtained show that *Vibrio* spp., in particular low virulence variants, are found in raw seafood consumed in Switzerland. Nevertheless, a potential risk must not be dismissed and further efforts are required in the development and standardization of molecular-based methods for monitoring purposes in seafood.

P2-124 Correlating Attachment and Infection of Norovirus Using an ELISA-based System

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Introduction: Human noroviruses cause approximately 58% of foodborne illnesses in the US. Molecular detection methods combined with the use of viral surrogates, including murine norovirus (MNV), are current tools for elucidating infectivity of environmental survival and inactivation of noroviruses by food processing. Recent studies on viral replication have revealed that MNV binds to sialic acid similarly to human norovirus attachment to histo-blood group antigens.

Purpose: A method was developed to assess norovirus attachment through binding to carbohydrate host cell receptors in an ELISA, using MNV as a surrogate. To better understand attachment, data was correlated to infectivity determined by plaque assay for heat and high pressure processed (HPP) MNV.

Methods: ELISA plates were coated with sialic acid (SA) (10µg/mL) and untreated, heat-treated (50-80°C) or HPP-treated (200-500 MPa at 50°C) MNV was added followed by polyclonal chicken anti-MNV IgY antibody (1:50, 1:100). Bound anti-MNV antibodies were detected by alkaline-phosphate conjugated goat anti-chicken IgY antibody. The average OD405 of MNV-containing wells were divided by negative control wells and expressed as the 'P/N ratio'; values ≥ 2 were considered positive as documented in the literature. Negative controls included wells without SA, MNV or IgY. Infectivity of MNV following heat and HPP treatments was determined using the plaque assay. Samples were tested in triplicate.

Results: HPP treated MNV attachment to SA significantly ($P < 0.05$) decreased with increasing levels of viral inactivation as observed with plaque assay; the P/N ratios at 200 and 500MPa were 3.65 ± 0.10 and 2.10 ± 0.06, respectively which corresponded to a 1.8 ± 0.70 and 6.29 ± 0.29 log PFU/mL inactivation. Conversely, heat treated MNV attachment to SA increased as virus inactivation levels increased; P/N at 50 and 80°C was 2.10 ± 0.32 and 3.50 ± 0.64, respectively. Polyclonal anti-MNV IgY was shown to bind to all treated MNV samples on ELISA plates coated with virus. These findings indicate potentially different effects on the viral capsid due to different food processing methods.

Significance: This ELISA-based assay highlights the potential to use viral interactions with host-cell carbohydrate receptors as a means to analyze norovirus infectivity and to further assess norovirus interactions with host cells.

P2-125 ST4, A Genetic Fingerprint for Meningitic Strains of *Cronobacter sakazakii* as Shown by Multilocus Sequence Typing

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Introduction: The *Cronobacter* (former *Enterobacter sakazakii*) is a newly recognized genus within the *Enterobacteriaceae*. Due to its link with neonatal and infant infections improved detection methods have been developed and microbiological criteria updated. Recent whole genome sequencing and comparative genomic hybridization microarray analysis (Kuvcerova et al., 2010 and 2011) has supported the use of multilocus sequence typing for further determining the diversity of *Cronobacter* and whether virulence varies between species or clones.

Purpose: A 7-loci multilocus sequence typing (MLST) scheme was developed to study the diversity of *Cronobacter* and serve as a robust, reliable, easily accessible online characterization tool. The method was applied to a wide collection of *Cronobacter* isolates to determine the relatedness of virulence (as determined by clinical source) with *Cronobacter* species and sequence type.

Methods: 175 strains representing all *Cronobacter* species were chosen for investigation. These strains were temporally, geographically and source defined. The *Cronobacter* MLST scheme based on seven protein coding genes (*atpD, fusA, glnS, gltB, gyrB, infB,* and *ppsA*; 3036 nt) was applied as described by Baldwin et al. (2009; http://www.pubMLST.org/cronobacter). Phylogenetic trees were constructed using the neighbor joining method, and sequence types (STs) analyzed for strain inclusion according to origin. Selected strains were investigated for in vitro virulence assessment using attachment and invasion of CaCo2 human intestinal cells and rat brain cells.

Results: A total of 55 defined sequence types (ST) were obtained across the whole *Cronobacter* genus. Clinical isolates of *C. sakazakii* were allocated to only 3/23 *C. sakazakii* STs. It was notable that meningitic strains were almost exclusively in ST4. There was no significant variation between STs in their attachment to human intestinal cells. However ST4 strains were more invasive of the rat brain cell line than the other clinical STs (1 & 8).

Significance: C. sakazakii (and other *Cronobacter* species) can be isolated from a wide range of sources; clinical and environment, and foods. MLST has revealed that clinical strains are only in 3/23 of the defined STs, and that meningitic strains are almost exclusively in ST4. Non-meningitic clinical strains were in ST8. Therefore the ability of *C. sakazakii* to cause meningitis is associated with a narrow range of strains and has a clonal basis. ST4, and to a lesser extent ST1, serve as a genetic fingerprint for meningitic *C. sakazakii*.

P2-126 Characterization of the Adhesive Organelles of the Neonatal Meningitis Agent, *Cronobacter* spp.

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Introduction: Cronobacter spp., previously classified as a *Enterobacter sakazakii*, are emerging opportunistic foodborne pathogens that cause infections of meningitis, necrotizing enterocolitis, and septicemia, particularly among neonatal infants and elderly persons. Typical vehicles of infection are temperature-abused, contaminated infant formula and dried food products, and these organisms have been cultured from a variety of food-production environments. The genus is comprised of six species groups, *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. dublinensis*, *C. muytjensii*, and a *C. genomospecies* 1, based on a polyphasic genetic and phenotypic scheme.

Purpose: For many meningitis-causative bacterial pathogens, initial adhesion to epithelial cells is mediated by surface organelles, such as type IV pili in *Neisseria meningitis*. In this study, we examined a large collection of *Cronobacter* spp. isolates using PCR to characterize the adhesive organelles of the six species groups.

Methods: We analyzed two *Cronobacter* spp. genomes, *C. sakazakii* BAA-894 and *C. turicensis* z3032, for adhesive organelles; i.e., fimbriae and curli. We then screened a collection of 231 *Cronobacter* spp. strains by PCR to determine the distribution of these genes.

Results: We found six chaperone-usher fimbriae, *in silico*, which were shared between *C. sakazakii* BAA-894 and *C. turicensis* z3032. In addition, *C. sakazakii* BAA-894 possessed three unique fimbriae, while *C. turicensis* z3032 possessed one unique fimbria and was curliated. When we screened our collection of *Cronobacter* spp. by PCR, we found 5 shared fimbriae and one additional fimbria that was only absent from *C. muytjensii* strains. Not all strains of *C. sakazakii* possessed the three unique chaperone-usher fimbriae present in *C. sakazakii* BAA-894 and some strains of *C. sakazakii* possessed the unique fimbria found in *C. turicensis* z3032. Thirty percent of *C. dublinensis* and *C. turicensis* strains were curliated, as well as all *C. malonaticus* and *C. genomosp.* 1 strains; No *C. muytjensii* or *C. sakazakii* strains were curliated.

Significance: Taken together, we propose that the total number of and, to a lesser degree, the type of adhesive organelles present play an important role in determining pathogenic potential of *Cronobacter* spp. strains.

P2-127 Heat Adaptation and Survival of *Cronobacter* spp. (formerly *Enterobacter sakazakii)*

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Introduction: Cronobacter spp. (formerly *Enterobacter sakazakii*) are Gram-negative opportunistic pathogens linked to cases of rare and potentially fatal neonatal diseases including, but not limited to meningitis, necrotizing enterocolitis and septicemia. The most susceptible population are immunoompromised and underweight infants. *Cronobacter* spp. are particularly associated with powdered infant formula and its production environment.

Purpose: Adaptation to sub-lethal stress can confer increased survival capability on foodborne pathogens. The purpose of this study was to investigate the adaptive response of *Cronobacter* spp. to heat and compare the survival of heat adapted to unadapted cells.

Methods: Five different isolates, representing 3 different *Cronobacter* spp., were adapted at 46°C for 30 min and subjected to a lethal stress at 52°C. Survivors were estimated by plate count on nutrient agar. Survival of adapted and unadapted cells during storage in dry powder was assessed. Gas Chromatography was used to measure the membrane saturated and unsaturated fatty acids.

Results: All strains showed increased survival upon adaptation. Survival was greater in milk-grown cells, but broth-grown cells showed a higher degree of adaptation. The survival potential acquired following adaptation was not transferred to survival in a dry environment. The ratio of membrane unsaturated to saturated fatty acids decreased, possibly resulting in a more rigid membrane in adapted cells.

Significance: Adaptation was demonstrated in *Cronobacter* spp. Physiological changes in the membrane during adaptation could explain the increased survival potential of heat-adapted cells.

P2-128 Evaluation of *flaA* Short Variable Region Sequencing, Multilocus Sequence Typing and Fourier Transform Infrared Spectroscopy in Discrimination of *Campylobacter jejuni* Strains

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Introduction: As *Campylobacter* contamination of food continues to constitute a serious human health risk, much effort is devoted to improving intervention and control strategies. Characterization of *Campylobacter* plays a key role in defining these preventive measures to reduce the burden of human infection. Revealing the significance of various reservoirs, understanding the epidemiology and infection dynamics in food production animals, the survival throughout the production chain and important sources for human infection, all rely on the possibility of discrimination on strain level.

Purpose: Discriminatory and robust characterization methods are needed for improved understanding of the dynamics of foodborne *Campylobacter* infections and the epidemiology of *Campylobacter* in the primary animal production.

Methods: The present study describes the first-time evaluation of the strain discriminatory potential of characterization methods *flaA* short variable region (SVR) sequencing and Fourier transform infrared (FTIR) spectroscopy on a collection of 102 epidemiologically related or unrelated *C. jejuni* field isolates. FTIR spectroscopy has not previously been described for characterization and subtyping of *Campylobacter*. A subset of isolates, initially discriminated by *flaA* SVR sequencing, was further subjected to multilocus sequence typing (MLST).

Results: It was found that *flaA* SVR sequencing had a slightly higher discriminatory power than FTIR spectroscopy, as indicated by a Simpsons diversity index of 0.94 and 0.92, respectively. Likewise, the results indicated that FTIR spectroscopy is indeed suitable for characterization of *Campylobacter*. Cluster analysis grouped the isolates in 6 clusters based on *flaA* SVR sequences and 9 clusters based on the FTIR spectroscopy profiles. Furthermore, the cluster analysis of *flaA* SVR sequences, MLST and FTIR spectroscopy profiles showed an unexpectedly high degree of congruence, assigning the isolates to similar cluster structures.

Significance: In conclusion, FTIR spectroscopy can be applied, not only for identification of *Campylobacter*, but also for further characterization and subtyping, and the high discriminatory potential of both *flaA* SVR sequencing and FTIR spectroscopy render them suitable for screening of large numbers of strains.

P2-129 Rapid Quantification of Viable *Campylobacter* from Chicken Carcasses, Using Real-time PCR and Propidium Monoazide Treatment, as a Tool for Quantitative Risk Assessment

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Introduction: The introduction of quantitative real-time PCR (Q-PCR) has enabled a faster, more sensitive and less labor-intensive quantification of foodborne pathogen bacteria. Q-PCR methods for foodborne *Campylobacter jejuni* and *C. coli* in poultry, which is recognized as an important source of human *Campylobacter* infections, have been published. However, since control strategies mostly focus on reduction of the number of bacterial cells on the chicken carcass, the usefulness of these Q-PCR methods for risk assessment could be limited, as they detect all *Campylobacter* present in a sample, including the dead cells.

Purpose: To enable rapid and reliable quantification of only viable foodborne *Campylobacter* bacteria.

Methods: A new and rapid quantitative protocol was developed based on the combination of Q-PCR with simple propidium monoazide (PMA) sample treatment. In less than 3 h, this method generated a signal from only viable, and viable but nonculturable (VBNC) *Campylobacter* bacteria with an intact membrane. The method's performance was evaluated by assessing the contributions to variability by individual chicken carcass rinse matrices, species of *Campylobacter*, and differences in efficiency of DNA extraction with differing cell inputs. The method was compared with culture-based enumeration on 50 naturally infected chickens.

Results: The cell contents correlated with cycle threshold (*CT*) values ($R^2 = 0.993$), with a quantification range of 1×10^2 to 1×10^7 CFU/ml. The correlation between the *Campylobacter* counts obtained by PMA-PCR and culture on naturally contaminated chickens was high ($R^2 = 0.844$). The amplification efficiency of the Q-PCR method was not affected by the chicken rinse matrix or by the species of *Campylobacter*. No Q-PCR signals were obtained from artificially inoculated chicken rinse when PMA sample treatment was applied.

Significance: In conclusion, this study presents a rapid tool for producing reliable quantitative data on viable *Campylobacter* bacteria in chicken carcass rinse. The proposed method does not detect DNA from dead *Campylobacter* bacteria but recognizes the infectious potential of the VBNC state and can thereby be useful to assess the effect of control strategies and provide data for risk assessment.

P2-130 Perspectives of Real-time Monitoring of *Campylobacter* and *Salmonella* Infections in Free-range Geese for Source Tracing of Human Cases and Risk Mitigating Interventions

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Introduction: Free-range domestic poultry flocks represent challenges in terms of reducing risk of foodborne transmission of *Campylobacter* and *Salmonella* to consumers but also offer unique opportunities to study interference with environmental reservoirs and epidemiological dynamics within flocks.

Purpose: To reveal the feasibility of real-time monitoring of colonization with *Campylobacter* and *Salmonella* in terms of understanding epidemiological dynamics in free-range goose flocks, improvement of traceability of strains from primary poultry production to human outbreak, and assessment of possibilities of risk-mitigating intervention in response to colonization dynamics in management and slaughter planning.

Methods: A complex pattern of splitting and merging of flocks of free-range geese was followed throughout the life of the flocks by collection of droppings and cultivation for *Salmonella* and *Campylobacter*. Isolates of *Salmonella* were identified to the level of serotype and phage type and isolates of *Campylobacter* were preliminarily identified to the level of species by a discriminatory PCR.

Results: While colonization with *Salmonella* tended to decline in the flocks, introduction of *C. jejuni* resulted in permanent colonization in all individuals, and *C. coli* was also frequently introduced to the flock. Colonization with *Salmonella* followed different patterns in different flocks. In one flock, all individuals became infected within one week at 2 to 4 weeks of age with a single strain of *S.* Enteritidis, which once it was introduced, persisted in the flock and from levels below the detection limit was transiently detected again in feces during an incidence of pneumonia in the flock. In another flock a strain of S. Mbandaka became introduced to the flock at 9 weeks of age but it did not spread aggressively. In this flock a multiplicity of serovars transiently appeared at 27 weeks of age.

Significance: The data illustrate the challenge in tracing outbreaks of zoonotic pathogens to primary production. The differences in epidemiology of *Salmonella* in the flocks suggest that risk mitigation in organically-raised poultry flocks based on semi-continuous monitoring is feasible. Monitoring might include control of other infections as well and adequate interventions include relocation of flocks and planning of slaughter time in relation to the course of *Salmonella* infection. However, quantification of colonization in conjunction with real-time monitoring is required to reveal if this is also a possibility for *Campylobacter*.

P2-131 In vitro Assessment of Temperature- and pH-dependent Growth Patterns of *Campylobacter jejuni* and *coli*

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Introduction: Campylobacter spp. are among the main bacterial causes of acute gastroenteritis worldwide. Despite their fragile nature, they survive in the environment and food chain, likely overcoming several stressful challenges. However, little is known about their response to adverse conditions.

Purpose: This study was conducted to investigate the survival of *Campylobacter jejuni* and *coli* at various temperatures (32, 37, 42, and 47°C) and pH ranges (5, 7, and 9) in laboratory media.

Methods: Strains of *C. jejuni* and *C. coli* were cultured separately in tryptic soy broth (TSB). In order to adapt bacteria to a mild stress, broth cultures were further inoculated in TSB at pH 6 and 8. After 24 h, the pH 6 culture was inoculated in TSB at pH 5, while the pH 8 culture in TSB at pH 9. Aliquots of these broths were incubated at 32, 37, 42 and 47°C and a pH 7 culture was used as control. Samples were taken at 0, 2, 4, and 24 h, spread plated onto Campy Cefex agar, and incubated for 48 h at 42°C.

Results: Extreme thermal and pH conditions (i.e., 32 and 47°C, pH 5 and 9, respectively) resulted in variable behavior of *Campylobacter* spp. Although *C. jejuni* and *C. coli* populations at pH 5 and 9 were significantly ($P < 0.05$) lower than those at pH 7, the survival populations still remained high at approximately 6 $\log_{10}$ CFU/ ml. Temperature (37 and 42 °C) did not affect ($P > 0.05$) the growth patterns of *Campylobacter* spp. and no interactions between strain, pH, incubation temperature, and sampling time were detected.

Significance: Results suggest that *C. jejuni* and *C. coli* do not respond well to multiple stresses, but have the ability to adapt to low and high pH. Therefore they can resist typical sanitation practices and persist in the environment leading to human illnesses.

P2-132 Analysis of *Campylobacter jejuni* Whole Genome DNA Microarrays: Significance of Prophage and Hypervariable Regions for Discriminating Isolates

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Introduction: Campylobacter jejuni is a major cause of gastroenteritis in humans and is carried in many common food animals. In order to reduce human infections, a better understanding of *Campylobacter* epidemiology is needed. Identifying genes that enable discriminating between isolates is an important factor in filling this need. A useful technique for this purpose is comparative genome indexing (CGI) using whole genome DNA microarrays.

Purpose: The objective of this study was to use CGI to identify the genes that were most significant for discriminating isolates of *C. jejuni* from humans, chickens, and beef cattle.

Methods: A geographically diverse population of 95 *C. jejuni* strains was selected from a collection of human, cattle and chicken isolates. Genomic DNA from each isolate was labeled and hybridized to microarrays composed of *C. jejuni* strains NCTC11168 (human; UK, 1980) and RM1221 (chicken; U.S., 2000) genes. The SAS program was used to analyze the presence or absence of genes and determine which variable genes were most informative.

Results: Statistical analyses of whole genome data from 95 geographically diverse cattle, chicken and human *C. jejuni* isolates identified a total of 142 most informative (i.e., significantly variable) genes. Of this total, 125 (88%) belonged to genomic prophage and hypervariable regions. Prophage and hypervariable genes were identified in isolates from all 3 hosts but were especially common in human isolates.

Significance: The significance of genomic prophage and hypervariable regions in determining *C. jejuni* isolate genomic diversity is emphasized by these results. These genes should prove useful in the development of a more efficient genotyping system for *C. jejuni* as well as furthering our understanding of the epidemiology of this major foodborne pathogen.

P2-133 Enhancement of PCR Amplification Capacity by a Simple Sample Preparation Procedure for the Detection of *Campylobacter jejuni* in a Blood-containing Enrichment Broth

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Introduction: A scorpion probe-based real-time PCR assay was developed for the quantitative detection of *Campylobacter jejuni* in foods after enrichment in a blood-containing enrichment broth which is used as China National Standard (GB) culture media for the detection of *C. jejuni* in China. The specificity of the assay for *C. jejuni* was demonstrated with a diverse range of *Campylobacter* species, related organisms, and unrelated genera. The assay was sensitive enough to detect 3–4 log colony-forming units (CFU)/mL of the target pathogen in pure broth culture. However the amplification capacity of PCR can be dramatically reduced or blocked by the presence of PCR-inhibitory substances naturally existing in blood, mainly heme and leukocyte DNA.

Purpose: The objectives of this study were: 1) to develop scorpion probe-based real-time PCR assay for the detection of *C. jejuni* in foods; and 2) to enhance the amplification capacity reduced or blocked by the presence of the PCR-inhibitory substances in blood.

Methods: A PCR template preparation method was applied to 1mL enrichment blood-containing broth. The purified DNA solutions were then transferred to PCR tubes in 30 µL aliquots and placed in the BAX® system cycler/detector, and a full process was run on Q7 instrument. The PCR template preparation methods were evaluated and compared with BAX® PCR test kit method.

Results: Parallel comparison on the enrichment samples showed 3–4 log (≥1000 times) enhancement of the sensitivity by PCR template preparation method as comparing with test kit method which does not have DNA purification procedure to remove PCR inhibitors in blood.

Significance: This approach demonstrates that PCR template preparation on blood-containing samples can significantly increase the sensitivity of the PCR assay. It can also apply to other difficult food matrices prior to PCR to remove the inhibitory substances and enhance PCR amplification capacity for a reduced time-to-result.

P2-134 Influence of Temperature and Cell-free Supernatant of *Staphylococcus aureus* on Biofilm Formation by *Listeria monocytogenes* on Stainless Steel Surfaces

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Introduction: The ability to form persistent biofilms makes the foodborne pathogen *Listeria monocytogenes* a hazardous contaminant in food processing environments and raises public health concern. Biofilms are composed of microorganisms attached to a surface encased in a self-produced extracellular polymeric substance matrix. Bacterial adhesion depends on physicochemical properties of the environment, surface and bacteria. Recently it was discovered that thermonuclease from *Stahpylococcus aureus* affects biofilm maturation.

Purpose: In this work, biofilm formation by *L. monocytogenes* on AISI 304 stainless steel surface was studied at different temperatures and the role of thermonuclease as a possible anti-biofilm strategy was tested.

Methods: Biofilms were obtained on the surface of stainless steel coupons vertically clamped on a stainless steel base placed in a beaker containing Brain Heart Infusion broth (BHI), with incubation at 37°C or 25°C and stirring of 50 rpm. *L. monocytogenes* was cultured in the presence and absence of cell-free supernatant of *S. aureus* with thermonuclease activity. The number of adhered cells was determined after 24, 48 and 72 h of biofilm formation and expressed as log CFU/cm^2.

Results: L. monocytogenes adhered and formed biofilms on stainless steel surfaces, with counts of 5.9 log CFU/cm^2 after 24 h of incubation at 37°C and decreasing numbers after 48 and 72 h (4.8 and 4.1 log CFU/cm^2, respectively). At 25°C a population of ca. 5.1 log CFU of *L. monocytogenes* per cm^2 was observed from 24 h up to the end of study, indicating a possible role for flagella and motility in biofilm formation. In the presence of *S. aureus* cell-free supernatant with thermonuclease activity, the formation of biofilms by *L. monocytogenes* was completely inhibited over 24 h of incubation at 37°C.

Significance: A variety of antimicrobials are typically produced by bacteria, including bacteriolytic enzymes, low molecular weight polypeptides and bacteriocins, which need to be further studied for controlling and/or preventing biofilm formation by foodborne pathogens.

P2-135 Modeling *Staphylococcus aureus* Growth and *Enterotoxin* Productions in the Chicken Slaughtering Process

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Introduction: Staphylococcus aureus is a significant cause of avian disease including commercially grown poultry. Its presence in processed carcasses may thus contaminate foods that are intended to be consumed by the general public. Poultry processing plants offer ideal environments for the survival and transmission of various commensal, spoilage, and potentially pathogenic bacteria thus capable of impacting the human food chain with profound results. In particular, Staphylococci, is one of the most predominant bacterial organisms present during the slaughtering and processing of poultry. Enterotoxin-producing *S. aureus* is the most common cause of foodborne human illness throughout the world.

Purpose: The purposes of this study were to investigate the quantification of *S. aureus* in the poultry slaughtering process, and to develop a growth model for enterotoxin production of *S. aureus* in the slaughtering process, thus creating a tool for assessment of food safety risk.

Methods: This study was conducted to evaluate the microbiological quality of poultry carcass samples from different slaughtering processes in large scale (>50,000 chicken/day) slaughtering houses. A "whole bird rinse" technique was used to analyze the incidence of microorganism on poultry carcasses after being scalded, eviscerated, generally chilled and packaged. Using the Gompertz and Barnyi models as the primary growth model of *S. aureus*, our research team compared results with those of an improved model, one which incorporated poultry processed at constant low temperatures.

Results: The levels of contamination in the chickens analyzed were quite low. Poultry carcasses after scalding showed higher incidence (10^3 CFU/mL) among the other carcasses. The modified model and the Baranyi model each more accurately described the early exponential phase of the growth curve than the previous model, at constant temperatures from 14 to 36.5°C. The amount of toxin in carcasses increased linearly with the passage of time from the moment the cell population reached about 10^5 CFU/ml. The rate of toxin production linearly increases at all temperatures between 14 and 32°C.

Significance: These results showed that the modified model and toxin production algorithm might be useful tools for modeling bacterial growth and enterotoxin production of *S. aureus* in the poultry slaughtering process, and thereby offer a valid assessment of risk for food safety for the general public.

P2-136 Systematic Ecological Investigation of *Bacillus* Species

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Introduction: Bacillus cereus is the most prevalent pathogenic *Bacillus* species found in foods. However, other *Bacillus* species, particularly *B. subtilis, B. licheniformis, B. brevis, B. pumilus* and *B. thuringensis*, have also been recognized as food poisoning bacteria of increasing concern, with reports of outbreaks of diarrheal or emetic food poisoning.

Purpose: This study aimed to systematically examine the microbial ecology of *Bacillus* species in rice products, commonly associated with *Bacillus* emetic food poisoning.

Methods: Bacillus species were isolated from rice products using centrifugation-plating and conventional spread-plating methods. The diversity of the isolates at the subspecies level was investigated using the RAPD-PCR typing technique and the toxigenic potential of isolates was determined by molecular and immunological analysis.

Results: RAPD-PCR results showed broad diversity among the strains and revealed some associations among isolates from the raw and the cooked rice samples, at the genotypic level. A comparatively greater diversity of strains was observed among isolates from raw rice than those from cooked rice and, generally, different RAPD profiles were found among isolates from raw rice and cooked rice, with few common to both types of rice. Toxin production and potential toxigenicity of the isolates were also investigated. The results revealed that other *Bacillus* species outside the *B. cereus* group were shown to produce emetic toxin.

Significance: The information generated, and systematic comparison of *Bacillus* species in raw and cooked rice samples, can be applied to establish risk assessment of bacilli in rice products.

P2-137 PCR Signals from Bacterial Pathogen-associated Genes in Beef Products as Process Control Indicators

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Introduction: Bacterial pathogens such as *Escherichia coli* O157 and *Salmonella* can be found on raw beef products. Testing for genes associated with these organisms can serve to monitor process control during the processing of beef products and signal sanitation failures sooner than methods based on culturing indicator microorganisms.

Purpose: Often the results of tests screening for pathogens must be known before the product is shipped; therefore, rapid methods are essential. Signals from PCR screening for pathogenic determinant genes provide an indication when sanitary practices may not have been adequate to prevent beef product contamination.

Methods: Samples of trim, carcasses swabs, ground beef and variety meats were analyzed from beef product manufacturing plants during Calendar Year 2009. Analysis for pathogenic determinant genes was performed using PCR and targeted *Salmonella*-specific genes, and *rfb, eae, stx*$_1$ and *stx*$_2$ genes of *E. coli* O157. In addition, an antibody-based linear flow method was used to detect the presence of the O157 antigen. The frequency of signals produced from these genes was compared with variables such as the month, the day of the week and the percent lean of the product.

Results: Samples (n= 681,991) were analyzed from trim (93.3%), ground beef (3.1%), carcasses (0.6%) and variety meats (3.1%) collected in four groups of plants (n=17). The average number of signals per sample varied by day of the week from 0.41 (Thu) to 0.47 (Tue), a significant difference ($P < 0.05$). When examined monthly, the average number of signals per sample (ANSS) was 0.44 and ranged from 0.30 (Aug-Sep) to 0.72 (Jun). The ANSS for trim varied from 0.62 for 50-59% lean to 0.29 for 90+% lean. Frequencies of positive samples ranged from 1.06% for the *sal*$_1$ target to 15.1% for the *stx*$_2$ gene. Some signals were correlated: *stx*$_1$ and *stx*$_2$ ($r = 0.85$), and *eae* and *stx*$_1$ or *stx*$_2$ ($r = 0.67$).

Significance: As PCR results signaling the possible presence of pathogens on beef products are rapidly obtained (~12 hours), and are more frequently positive than the isolation of pathogens, these signals can be used as a sensitive and convenient measure of process control with regard to sanitation. Furthermore, beef processors might consider employing a higher level of interventions when contamination is more frequent.

P2-138 The Effect of the Presence of the Plant Pathogen *Erwinia tracheiphila* on Internalization of S*almonella* Poona in Cantaloupe Fruits

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Introduction: Outbreaks of salmonellosis have been associated with the consumption of cantaloupes. *Salmonella* can reside on cantaloupe rind surfaces, but whether it can enter interior fruit tissues is still unknown.

Purpose: Our objectives were to determine whether *Salmonella* can enter cantaloupe fruits through natural cracks formed on rind during net development and to evaluate whether the presence of the plant pathogen *Erwinia tracheiphila (Et)* affects the likelihood of *Salmonella* internalization.

Methods: Young cantaloupe fruits having freshly formed rind cracks were spot-inoculated with peptone solution, *S.* Poona, or a mixture of *S.* Poona and *Et* (20 µl each; 10^7 CFU/ml). Samples of rind and the fruit tissues immediately below were collected 7 days post-inoculation (dpi) and at fruit maturity (about 19 dpi). After enrichment in a Universal Pre-enrichment Broth, aliquots were grown in *Salmonella*-selective broths (Tetrathionate and Rappaport Vassiliadis) and streaked on XLD plates, from which black colonies were selected for PCR confirmation of *Salmonella* using *Salmonella invA*-based primers.

Results: S. Poona survived on the rind surface from the time of inoculation to fruit maturity in treatments of *S.* Poona alone or of the *S.* Poona-*Et* mixture. The mixture, but not *S.* Poona alone, caused water-soaked lesions resembling those caused by *Et* alone, on some fruits, and *S.* Poona was recovered from interior mesocarp of 2 fruits bearing lesions (one 7 dpi, one 19 dpi). These 2 fruits represent 17% of those receiving the *S.* Poona-*Et* mixture. The results suggest that *Et* may facilitate the internalization of *Salmonella* in developing cantaloupe fruits.

Significance: This preliminary study is the first report of *Salmonella* internalization in the edible portion of cantaloupe fruit. Uptake of human pathogens through naturally occurring rind cracks may be possible in the field, when fruits may come into contact with contaminated substrates.

P2-139 Efficacy of Alfalfa Seed Sanitation Using a Commercial Compressed Air Bubbler Seed Washer as a Function of Sanitizer Concentration

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Introduction: Sprouts have been the source of many outbreaks of foodborne illness. The outbreaks are generally thought to arise from contaminated seed. Therefore, FDA guidance directs sprout growers to sanitize alfalfa seeds with calcium hypochlorite prior to sprouting.

Purpose: FDA guidance is not clear regarding the basis for calculating chlorine concentration and whether pH should be controlled. Furthermore, it is difficult to reproduce seed sanitation in a laboratory setting. The purpose of the present study was to duplicate the sanitation apparatus used in commercial sprouting operations, and use a consistent protocol to evaluate how changes in conditions affected sanitation efficacy when applied to seed loads inoculated with *Salmonella* and *E. coli* O157:H7.

Methods: Inoculated seeds were sanitized using commercial compressed air bubbler seed washers. Chlorine concentrations ranging from 2,000 to 20,000 ppm (corresponding to 3,000 to 30,000 ppm as calcium hypochlorite, $CaClO_2$) were evaluated with and without the addition of citric acid to lower the pH. In addition, the commercial product FIT2 was tested.

Results: Reductions in *Salmonella* spanned 2.14 to 4.25 log MPN/g for non-buffered chlorine concentrations of 2,000 to 13,000 ppm, respectively. Using 20,000 ppm non-buffered chlorine did not improve the efficacy. Buffering the chlorine gave corresponding low and high reductions of 2.77 and 4.51 log MPN/g. At high concentrations (20,000 ppm) buffered chlorine was unstable due to disproportionation reactions. Corresponding non-buffered reductions in *E. coli* O157 levels were 2.68 to 4.66 log MPN/g, respectively, and for buffered chlorine 2.92 and 7.62 log MPN/g, respectively. Water alone reduced levels of *Salmonella* spp. by almost 1 log, and *E. coli* O157 by almost 2 logs. The FIT2 treatment gave results similar to treatment by water washing alone. None of the treatments had an unacceptably adverse effect on seed germination rates.

Significance: This study confirmed that seed washing and sanitizing alone cannot eliminate these two pathogens. However, if other interventions are available it can play a role in a multiple-hurdle microbial intervention approach. Furthermore, as recommended by the FDA seed sanitation can be combined with microbial testing of spent sprout irrigation water.

P2-140 The Effect of Temperature History on the Ability of Proteolytic Strains of *Clostridium botulinum* to Produce Toxin in Extended Shelf-life Foods

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Introduction: Refrigerated carrot juice was responsible for a botulism outbreak in the US and Canada in 2006 that resulted in 6 cases and 1 death. It is believed that the outbreak was the result of multiple factors, including holding the juice at an elevated temperature for a period of time just prior to filling. Botulism continues to be a concern in extended shelf-life refrigerated foods.

Purpose: To identify and avoid storage combinations of time and temperature that could stimulate spores of *C. botulinum* to germinate and produce toxin under environmental conditions where these spores would normally remain dormant.

Methods: C. botulinum type A (CDC-CR1) and proteolytic B (Mush 3B) spores were exposed to various time-temperature combinations (27+1 °C for 3 h, 37 °C for 1 h, 80 °>C for 10 min, 80°C for 15 s and 93°C for 30 s) then inoculated at levels of 10^2 and 10^4/mL to determine the effects of time-temperature on germination and toxin production. Spores from each treatment were inoculated into the TPGY tubes of incrementally varying pH and incubated anaerobically at 37+1°C then tested for toxin presence.

Results: The minimum pH for toxin production by CDC-CR1 in the media used was 5.4 for 10^4 and 5.4–5.6 for 10^2 spores/mL and 5.4–5.6 and 5.7-5.8 for 10^4 and 10^2/mL for Mush 3B, respectively. At inoculum levels of 10^4 spores/mL, pretreatment history combinations of 27+1°C for 3 h and 80 °C for 15 s decreased the minimum pH necessary to support toxin production; however, the decrease in pH was reproducible yet not significant. The effects of the treatments were greater at the higher inoculum.

Significance: Knowing the effect of various time-temperature exposures at the processor and consumer will result in avoidance of potentially hazardous situations and avoid botulism outbreaks and demonstrate why a food suddenly supports toxin formation when it has previously had a good safety record.

P2-141 The Use of a Global Web-based Foodborne Pathogen Annotated Tracking Resource Network (PATRN) System for the Integration, Analysis and Visualization of Four *Cronobacter sakazakii* Isolates from Two Recent Neonatal Meningitis Cases

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Introduction: The Pathogen Annotated Tracking Resource Network (PATRN) system is a FDA-sponsored application of a web-based resource (www.patrn.net) for the integration of heterogeneous assay results from outbreak and historical isolate data that can be analyzed comparatively and visualized. Genome sequencing of two *Cronobacter* strains revealed that they harbor RepFIB plasmids identified as pESA3 and pCTU1. In silico analysis of these plasmids showed that both carry a single replicon gene, repA, as well as two iron-acquisition systems (eitCBAD and iucABCD/iutA). Additionally, pESA3 carries a cpa (*Cronobacter* plasminogen activator) gene, and a type six secretion system (T6SS) locus, while pCTU1 contains a filamentous hemagglutinin/adhesin gene cluster (FHA locus).

Purpose: As a use case for PATRN's application in the real-world of foodborne outbreaks, we analyzed four isolates from two cases of neonatal meningitis that were associated with the consumption of contaminated infant formula which occurred in the United States during the summer of 2010.

Methods: The isolates were identified phenotypically and by using a *Cronobacter* species-specific PCR method based on the rpoB gene. PFGE and optical mapping analyses were performed on these isolates and they were screened by PCR using primers targeting the repA, LPS, eitA, iucC, cpa genes, T6SS cluster genes, fhaB and related flanking regions to determine the distribution of these traits.

Results: The four isolates were identified as *Cronobacter sakazakii* and possessed the O2 LPS molecular serotype determinant. PFGE analysis showed that there were three band differences among the isolates (97% relatedness) which by optical mapping were shown to be due to the presence of three indel regions (59, 37, and 14 kb). PCR analysis showed that they possess multiple RepFIB plasmid-borne loci: repA, eitA, iucC, cpa and a T6SS locus, but not fhaB. This information was uploaded into PATRN to determine whether isolates with similar traits were present in 231 curated *Cronobacter* spp. isolates. Hierarchical cluster analysis showed that the four outbreak isolates matched two historical isolates. One of which, Jor172 was isolated from an unknown spice sample in Jordan in 2008; and the other, E772 was isolated from a milk powder sample in France in 2006.

Significance: The combination of molecular methods with bioinformatics tools housed in PATRN was highly effective in connecting related isolates from two recent cases to known archival isolates. PATRN will augment the Agency's food safety emergency response activities and will enhance FDA's ability to limit public health consequences of foodborne disease outbreaks.

P2-142 Inhibitory Effect of Foodborne Pathogens during the Fermentation of Dongchimi

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Introduction: Kimchi is a Korean traditional fermented food. Unlike many other kinds of kimchi, Korean water kimchi is called Dongchimi. It is also fermented with lactic acid bacteria. However, microbial characteristics and safety for Dongchimi are not much studied.

Purpose: The aim of this study is to investigate the survival of foodborne bacteria during the fermentation of Dongchimi.

Methods: Dongchimi samples were prepared by traditional recipe. Each sample was spiked with 7.41 log CFU/mL *E. coli* O157:H7 or 6.15 log CFU/mL *L. monocytogenes*. Dongchimi samples were fermented for 20 days at 10°C. The number of *E. coli* O157:H7, *L. monocytogenes*, and lactic acid bacteria were counted and pH and acidity measured at 1, 2, 3, 5, 7, 10, 15, 20 post-fermentation days (PFDs).

Results: E. coli O157:H7 and *L. monocytogenes* spiked in Dongchimi significantly decreased after 7 days. *E. coli* O157:H7 and *L. monocytogenes* showed more than a 6 log reduction in 15 PFDs. Lactic acid bacteria reached 7.70~8.82 log CFU/mL at 5 PFDs. A significant change in pH and acidity was observed.

Significance: E. coli O157:H7 and *L. monocytogenes* were significantly reduced by the lactic acid-producing bacteria during the fermentation of Dongchimi. This study suggests that Dongchimi is a microbiologically-safe fermented food.

P2-143 Development of a 16S rRNA Library for the Identification of Foodborne Pathogens

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Introduction: DNA sequencing is an emerging method for the detection and characterization of food pathogens in a regulatory setting. The MicroSEQ ID Microbial Identification System presents a standardized method for this purpose, based on sequence variation within the 16S rRNA gene. Despite the high potential of this method, the current 16S rRNA reference library does not contain comprehensive sequence information for food pathogens.

Purpose: The purpose of this project was to obtain 16S rRNA sequence information for a range of bacterial isolates representing *Salmonella enterica*, *Shigella*, and *Escherichia coli* in order to improve the 16S rRNA reference library for the identification of food pathogens at the species or serotype level.

Methods: DNA extracts from 43 serotypes of *Salmonella enterica* and 52 isolates of *Shigella/Escherichia coli* were PCR-amplified and sequenced for a 500 base-pair region of the 16S rRNA gene using the Fast MicroSEQ ID 500 Bacterial Identification kit. DNA sequences with quality scores ≥ 35 and sequence recovery ≥ 80% were entered into a custom 16S rRNA reference library and phylogenetic analysis was conducted with neighbor-joining trees.

Results: Sequences meeting the parameters above were obtained for 40 *S. enterica* serotypes and 45 *Shigella/E. coli* isolates, and a custom reference library was successfully created. Neighbor-joining trees of the *S. enterica* sequences showed clear separation for 21 serotypes, including 11 of the top 40 disease-causing serotypes and the remaining 19 serotypes showed 9 distinct groupings of 2-4 serotypes each. Most isolates (n = 42) of *Shigella/E. coli* formed distinct species-specific groupings; however, there were several cases of species overlap, where variation within species was greater than between species.

Significance: The results of this study will contribute to the building of a comprehensive 16S rRNA sequence library for the identification of bacterial pathogens in a regulatory setting.

P2-144 Antibiotic-resistant Enteric Bacteria Isolated from Animal Manure and Soils from Local Farms in Middle Tennessee

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Introduction: The use of antimicrobials in animal production results in the presence of drug resistant bacteria in our environment and has raised concern about transmission of zoonotic pathogens to humans. Although farmers benefit through less morbidity and mortality rate of the food animals, it has been suggested that the use of antibiotics in animal production causes antibiotic-resistant infections.

Purpose: The purpose of this study was to ascertain the profiles and patterns of antibiotic-resistant bacteria in animal manure and soil collected from local farms.

Methods: Samples of animal manure (n=34) and soil (n=43) were collected from farms and analyzed for antimicrobial resistance. Bacteria identification to species level was performed by commercially available identification kit, API 20E. *Salmonella* spp and *Escherichia coli* O157:H7 were isolated by using xylose-lysine-tetrathionate 4 agar and sorbitol MacConkey agar (cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l), respectively. Kirby-Bauer disc method was used to determine sensitivity of selected antimicrobial agents. Microbes were tested for sensitivity to ciprofloxacin, ampicilin, streptomycin, kanamycin, nalidixic acid, tetracycline, colistin, gentamicin, and cefoxitin.

Results: Our study indicated that cow manure and soils from local farms were contaminated with *Enterobacteriaceae* between 3.95 CFU/g to 6.93 CFU/g and 4.30 CFU/g to 9.85 CFU/g, respectively. *Enterobacteriaceae* showed resistance prevalence values reaching 5%, 32%, 45%, and 65% for antibiotics ciprofloxacin, colistin, streptomycin, and tetracycline, respectively. Among the microbes tested, no gentamicin resistance was observed. According to this study, animal manure and soils were contaminated with antibiotic- resistant *Escherichia coli* O157:H7, *Salmonella* spp, *Yersnia enterocolitica*, *Enterobacter cloacae*, *Klebsiella terrigena*, *Enterobacter sakazakii*, and *Flavimonas oryzihabitans*.

Significance: These data suggest that the farm environment is contaminated with antibiotic-resistant enteric bacteria. Further studies involving larger sample sizes over time are desirable to better monitor and evaluate the trend of frequency and antimicrobial susceptibility among microbes in farm environment.

P2-145 Fates of *Burkholderia thailandensis* in Acidic Conditions by Various Organic Acids Used in Ready-to-Eat Meat Formulations

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Introduction: Burkholderia pseudomallei has been known to be related to melioidosis with significant morbidity and mortality up to 90%, and it has recently brought attentions in food supply environments.

Purpose: This study evaluated the antimicrobial activity of meat processing-related organic acids on *Burkholderia thailandensis* (*B. pseudomallei* surrogate) under different water activities.

Methods: B. thailandensis KACC12027 (4 log CFU/ml) was inoculated in microplate wells containing tryptic soy broth pH-adjusted to 4, 5, 6 and 7 with ascorbic acid, citric acid, and lactic acid, and water-activity adjusted to 0.94, 0.96, 0.98 and 1.0 with NaCl, followed by incubation at 35°C for 30 h. The optical density (OD) of the samples was measured at 0, 3, 6, 12, 24, and 30 h at 595 nm to measure growth of *B. thailandensis*. All least squares mean comparisons among the interactions of treatments were performed with the pairwise t-test at alpha = 0.05.

Results: Growth of *B. thailandensis* was observed only at 1.0 of water activity. For ascorbic acid, *B. thailandensis* did not grow at less than pH 5.0, but *B. thailandensis* treated with lactic acid and citric acid showed bacterial growth even at pH 5. In addition, more growth ($P < 0.05$) of the bacteria treated with ascorbic and lactic acid was observed at pH 6 than at pH 7, and the growth of citric acid-treated *B. thailandensis* was higher at pH 5 than at pH 6 and 7. Antimicrobial effects of the organic acids on *B. thailandensis* were ascorbic acid > lactic acid > citric acid after incubation at 35°C for 30 h.

Significance: These results suggest that use of organic acids in meat processing-related formulation should be useful in decreasing the risk related to an emerging high risk bacteria (*B. pseudomallei*).

P2-146 Identification of Farm Practices Associated with the Presence of Psychrotolerant *Bacillus* Species and Related Sporeformers in Bulk Tank Milk

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Introduction: Sporeforming bacteria, *Bacillus* spp. and *Paenibacillus* spp., commonly found in farm environments and present in raw milk, are known to survive pasteurization and grow at refrigeration temperatures, thereby causing fluid milk product spoilage.

Purpose: Assess the overall presence of sporeformers in bulk tank milk from New York State dairy farms and identify farm management practices associated with resulting sporeformer counts.

Methods: One bulk tank sample was obtained and a management/herd health questionnaire was administered by a trained technician using a cross-sectional study design with 108 New York State dairy farms from May 2009 – June 2010. A portion of each bulk tank sample was spore pasteurized (176°F, 12 min) and taken for microbiological analysis on the initial day of pasteurization as well as 7 d, 14 d, and 21-d post-pasteurization storage at 6°C. Microbiological analysis was performed using the standard plate count for both the raw bulk tank samples as well as spore pasteurized samples, the preliminary incubation count and the psychrotrophic bacteria count for the raw samples, and the most probable number test on the spore pasteurized samples. After bacterial enumeration, farms were divided into two groups, designated as high (> 20,000 CFU/mL at 21-d post-pasteurization) or low (≤ 1,000 CFU/mL for all 21-d post-pasteurization).

Results: There were 68 farms categorized into the high group, with an average spore count of 5.14 mean log CFU/mL 21-d post-pasteurization. In the low group, there were 40 farms with an average spore count of 0.81 mean log CFU/mL 21-d post-pasteurization. Preliminary statistical analysis identified a significant association between the number of spores in pasteurized samples and 3 management practices associated with cleanliness (dirty udders, unclean housing areas, and parlor equipment maintenance). More detailed statistical analysis is ongoing to identify management practices that are associated with sporeformer loads in milk.

Significance: The results of the current study in addition to a future longitudinal study will distinguish seasonal trends in spore amounts on the two categories of farms, ensure correct categorization in high or low groups, and identify management practices which could be improved to ensure longer shelf-life, higher quality pasteurized fluid milk.

P2-147 Investigating Regulatory Compliance of the South African Informal Milk-producing Sector

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Introduction: The South African informal sector has grown considerably as a result of deregulation and socioeconomic changes, creating ample opportunity for employment and additional income to the informal sector. The current research assessed whether municipal health services at local government sphere are informed as to the extent of, and control over the informal milk-producing sector in their respective areas.

Purpose: The purpose of the empirical procedure was to solicit information regarding the nature and extent of compliance of milk produced by the informal sector and thus reflect on the effectiveness of regulatory procedures.

Methods: A questionnaire targeting 52 municipal health services managers at various metropolitan and district municipalities was conducted. Questions covered a wide range of aspects that focused on the effectiveness and capacity of local and provincial government to assess informal milk quality.

Results: Results indicated that a notable number of informal milk producers existed per metropolitan and district municipality under limited control by municipal health services. Furthermore, only one district municipality was authorized to allow the sale of raw milk in its area, whilst in most metropolitan and district municipalities the distribution of raw milk continues. At the time of the survey, a substantial number of metropolitan and district municipalities have not performed statutory Section 78 investigations to assess their ability to deliver municipal health services by way of capacity analyses.

Significance: The main finding of the study entails a clear indication that the municipalities responsible for informal milk provision largely lack the capacity and knowledge to perform effective monitoring and tracing of informal milk distribution and compliance. To this effect national and provincial government should be encouraged to support the capacity of municipalities to exercise power and perform their functions, in order to prevent the distribution of milk of poor quality to the wider community.

P2-148 Fate of *Listeria monocytogenes* in Indian Fermented Milk Products – Dahi and Buttermilk

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Introduction: Several pathogens including *Listeria monocytogenes* have been reported to grow and survive during the manufacture of fermented milk products like cheese and yoghurt.

Purpose: Evaluate the fate of *L. monocytogenes* during manufacture and subsequent storage of dahi and buttermilk (Indian fermented milk products).

Methods: Dahi was prepared by fermentation of heat treated milk to an acidity of 0.90 –0.95%. Dahi was mixed with water (50:50 wt/wt) and salt (0.7% wt/wt) and blended to obtain buttermilk. A five strain *L. monocytogenes* cocktail was either added to the milk along with the lactic culture (pre-fermentation contamination) or buttermilk (post-fermentation contamination) to achieve an initial concentration of ca. 3.5 or 6.5 log CFU/ml.

Results: L. monocytogenes population increase was minimal (0.15 –0.24 log CFU/ml) during fermentation, while significant reduction in the population was observed in dahi (2.55 to 2.77 log CFU/ml) during storage at 4°C. Lower reductions in *L. monocytogenes* population (1.59 and 1.18 log CFU/ml inoculated with low and high levels, respectively) were observed in buttermilk (pre-fermentation contamination) during storage at 4°C. The corresponding decreases were 1.19 and 0.89 during storage at 8°C. Irrespective of the inoculum level, *L. monocytogenes* population in post-fermentation contaminated buttermilk decreased by ca. 0.70 and 0.55 log CFU/ml at 4° and 8°C, respectively.

Significance: L. monocytogenes can survive during dahi manufacturing process, subsequent refrigerated storage, and in buttermilk during refrigerated storage. Good hygiene and sanitation should be followed during the preparation of dahi and buttermilk to reduce the risk of listeriosis.

P2-149 Survival of *Listeria monocytogenes* in Lassi, an Indian Fermented Sweetened Milk Beverage

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Introduction: Fermented milk products like cheese and yogurt have been implicated in *Escherichia coli* O157:H7 and *Listeria monocytogenes* associated foodborne illnesses.

Purpose: Survival of *L. monocytogenes* in lassi, an Indian fermented, sweetened milk beverage prepared from dahi during refrigerated storage (4 or 8°C) was evaluated.

Methods: Dahi was prepared by fermenting heat-treated milk to an acidity of 0.90-0.95%. Dahi was mixed with sugar syrup (60% wt./vol.) and water and blended to obtain lassi. A five-strain *L. monocytogenes* cocktail was added to milk along with lactic culture (pre-fermentation contamination) or lassi (post-fermentation contamination) to achieve an initial population of ca. 2.50 (low inoculum) or 5.75 log CFU/mL (high inoculum) in lassi. Changes in titratable acidity, pH, populations of lactic cultures and *L. monocytogenes* were determined in lassi during storage (4 or 8°C).

Results: L. monocytogenes reductions of 1.96 and 2.68 log CFU/mL were observed in lassi prepared from inoculated milk (pre-fermentation inoculation) with low and high inoculum levels, respectively during storage at 4°C. Corresponding reductions were 2.29 and 2.97 log CFU/mL during storage at 8°C. In post fermentation contaminated lassi, *L. monocytogenes* population in high inoculum samples reduced by 0.15 and 0.35 log CFU/mL at 4 and 8°C, respectively, while the reductions in lassi with low inoculum level were 0.62 and 1.13 log CFU/mL at 4 and 8°C, respectively.

Significance: L. monocytogenes can survive in lassi during refrigerated storage at 4 or 8°C. Good hygiene and sanitation should be adopted to minimize the risk of *L. monocytogenes*-related illness from lassi.

P2-150 Controlling *Listeria monocytogenes* and *Pseudomonas* spp. in Cottage Cheese Using a Combination of Calcium Lactate and Cultured Whey

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Introduction: Fresh cheeses, such as cottage cheese, are vulnerable to microbiological proliferation, leading to deteriorated products or even health risks. PuraQ Safe RS20P, an antimicrobial blend containing calcium lactate and cultured whey is a potential inhibitor for pathogens and Gram-negative spoilage bacteria.

Purpose: In this study, the antimicrobial effect of a calcium lactate/ cultured whey blend was evaluated against *Listeria monocytogenes* and *Pseudomonas* spp. in a "cottage cheese style" fresh cheese.

Methods: Cottage cheese samples were formulated with 0%, 0.15% and 0.3% PuraQ Safe RS20P. All samples were adjusted to a pH of 5.2 and subsequently inoculated with a cocktail of *Listeria monocytogenes* and stored at 40°F or inoculated with a cocktail of *Pseudomonas* spp. and stored at 45°F. All samples were stored for a maximum of 21 days.

Results: For *Listeria monocytogenes*, no growth was observed throughout the 21 days of storage when cottage cheese was formulated with 0.3% of the antimicrobial blend. For cottage cheese inoculated with *Pseudomonas* spp., a concentration level of 0.15% antimicrobial blend was enough for complete inhibition of growth during the 21 days of measurement. Rapid growth was observed for both the bacterial species in the control product without the antimicrobial blend.

Significance: With the current interest of the FDA in ensuring the food safety of dairy products (PMO requirements), this study shows that a combination of calcium lactate and cultured whey has the ability to suppress the growth of important pathogenic and spoilage bacteria in cottage cheese.

P2-151 Thermal Inactivation of *Salmonella* in Cheesecake

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Introduction: Foodborne illness attributed to *Salmonella* remains a significant public health concern in the United States as evidenced by the 2010 multi-state outbreak of *S.* Enteritidis associated with raw shell eggs. Whereas proper cooking of eggs is known to eliminate *Salmonella*, foods subjected to relatively mild heating or known to contain intrinsic properties providing thermal protection to *Salmonella* may pose a risk to consumers if prepared with contaminated ingredients. One example of such a food is cheesecake.

Purpose: This study investigated the time-temperature relationships required to inactivate both a 5-$\log_{10}$ and 7-$\log_{10}$ CFU/g inoculum of *Salmonella* when manufacturing cheesecakes.

Methods: Survival curves were generated by inoculating cheesecake batters with a two-strain cocktail of *Salmonella* [*S. enterica* (ATCC 29058) and *S.* Typhimurium (ATCC 13311)], holding the inoculated batters at three different temperatures (60°C, 65.6°C and 71.1°C) and direct plating onto both selective and non-selective agars over time.

Results: D-values at each temperature were then determined from the survival curves (D_{60C} = 14.6, $D_{65.6C}$ = 6.12 and $D_{71.1C}$ = 2.0) and a Z-value (10°) calculated. Predicted 5-$\log_{10}$ and 7-$\log_{10}$ CFU/g reductions of *Salmonella* were confirmed via direct challenge of cheesecake batter maintained at 65.6°C and 71.1°C using a five-strain cocktail [*S. enterica* (ATCC 29058), *S.* Enteritidis (ATCC 13086), *S.* Infantis (ATCC 51741), *S.* Tallahassee (ATCC 12002) and *S.* Typhimurium (ATCC 13311)] and subsequent analysis via direct plating and FDA BAM enrichment methodology.

Significance: This information may be useful for commercial manufacturers of cheesecake in the development of HACCP plans and validation of thermal processes, helping to ensure that the potential presence of *Salmonella* is eliminated during the baking process.

P2-152 Occurrence of *Escherichia coli* and *Staphylococcus aureus* on Dairy Farms in Hungary

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Introduction: Escherichia coli is part of the bacterial flora in feces of healthy dairy animals. Contamination of raw milk can easily occur during collection. *Staphylococcus aureus* is the most prevalent and economically significant pathogen causing intramammary infections in dairy ruminants.

Purpose: The primary objective of this work was to study the influence of farm size, housing conditions, milking method, pre-milking udder preparation, and teat disinfection on *E. coli* and *S. aureus* counts in raw bovine milk.

Methods: Seven large, four medium size, and nine small farms were enrolled in the research. The farms tested used deep litter, loose cubicle, or tie-stall housing systems. Milking methods included bucket milking, pipeline milking, and parlor milking. From each farm at each sampling time 50-ml bulk tank milk samples were collected which, cooled to 2°C, were immediately taken to the laboratory for microbiological examination. Along with various surface swab samples, a total of 1532 mammary quarter milk samples were also collected from mastitic cows on six large farms, and were subsequently enumerated for *S. aureus* and *E. coli*.

Results: The results showed that farm size had no significant effect ($P > 0.05$) on the *S. aureus* counts in bulk milk. It was found, however, that the milk produced by cows housed in cubicles had significantly lower ($P < 0.05$) *S. aureus* counts than did the milk batches produced in either tie-stall or deep litter housing systems. Milking conditions resulting in lowest *S. aureus* counts included pipeline milking and post-milking disinfectant teat dipping. In contrast, the majority of management and milking conditions tested appeared not to significantly affect ($P > 0.05$) the numbers of *E. coli* in bulk tank milk. The surface swab samples collected on large farms were of similar microbial quality, in terms of both *S. aureus* and *E. coli*, to those taken on small farms. The percentages of mastitic quarter milk samples positive for *E. coli* and *S. aureus* were 12.3 and 9.3, respectively.

Significance: It was concluded that the principal source of *S. aureus* and *E. coli* contamination of bulk tank milk was the infected udder of mastitic cows. *Acknowledgment:* Author L. Varga gratefully acknowledges research funding support from the National Development Agency of Hungary (Project No.: TÁMOP-4.2.1.B-09/1/KONV-2010-0006).

P2-153 Growth of *Staphylococcus aureus* in Probiotic Minas Fresh Cheese

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Introduction: Minas fresh cheese, one of the most consumed dairy products in Brazil, is a soft white cheese produced by enzymatic coagulation. Due to its high water activity, pH above 5.0 and low salt content, the presence of pathogens, such as *E. coli, S. aureus* and *L. monocytogenes* have been frequently reported. Currently probiotic Minas fresh cheese is commercially available in Brazil and beyond its health benefits to the consumer, the presence of probiotic bacteria might also be a hurdle to pathogen growth.

Purpose: The aim of this work was to evaluate the growth of *Staphylococcus aureus* during production and refrigerated storage of probiotic Minas fresh cheese.

Methods: Minas fresh cheese was produced with pasteurized milk, with the addition of *S. aureus* (previously isolated from a commercial Minas fresh cheese) and L. acidophilus NCFM. A control cheese was inoculated only with *S. aureus*. The populations of microorganisms were determined in milk, in cheese on the day of production and during 21 days of storage at 5°C. All cheeses were produce in triplicate and the results analyzed by ANOVA.

Results: S. aureus population increased significantly ($P < 0.05$) from 2.2 ± 0.4 log CFU/g (in milk) to 3.8 ± 0.5 log CFU/g (at the end of cheese production) and no significant difference ($P > 0.05$) was observed in relation to control. A same population of *L. acidophilus* was observed in milk and in cheese at the day of production (6.9 ± 0.4 log CFU/g). During 21 days of storage, the number of *S. aureus* remained constant in probiotic cheese (3.9 ± 0.5 log CFU/g) and also in the control cheese (3.3 ± 0.3 log CFU/g).

Significance: A significant growth of *S. aureus* occurred during probiotic Minas fresh cheese production while the population remained constant under refrigerated storage. *L. acidophilus* had no inhibitory effect on *S. aureus* growth in Minas fresh cheese.

P2-154 Prevalence and Antibiotic Resistance of Mastitis Pathogens Isolated from Dairy Herds Transitioning to Organic Management

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Introduction: Organic dairy production has increased worldwide. In the USA, dairy producers must follow strict guidelines that prohibit administration of antibiotics to animals that will become and/or produce food considered organic. Many studies have reported that reduced therapeutic and prophylactic use of antibiotics is related with decreased antimicrobial resistant bacteria. Decreasing pathogens resistant to antibiotics is of global concern in food production animals.

Purpose: The purpose of this study was to determine the change of intramammary infection (IMI) prevalence and antimicrobial resistance in mastitis pathogens isolated at the end of lactation and at parturition from cows in two herds transitioning to organic milk production.

Methods: Two commercial dairy herds in Randall, WA, USA, of approximately 50 lactating cows each were enrolled in this study. Milk samples from all four mammary quarters were aseptically collected from cows at the end of lactation and within a day of parturition, and from cows exhibiting clinical mastitis. The study was conducted during the last year of conventional dairy production, during the transition year, and during the first year of organic production. Antibiotic resistance testing was done using the modified Kirby-Bauer Method. Statistical analysis was performed using the SAS system.

Results: The most prevalent pathogen type to cause intramammary infection was CNS, and followed by *Streptococcus* spp., other than *Streptococcus agalactiae*, at parturition and dry-off. There was a significant increase in the zone diameter around the ampicillin, cephalothin, cloxacillin, and penicillin discs for the CNS by period, conventional vs. transition and organic phases of production ($P < 0.05$). The percentage of CNS isolates deemed resistant to β-lactam antibiotics decreased from the conventional period when compared to the organic period.

Significance: This is the first study documenting a change of antibiotic resistance that occurs during the longitudinal transition from conventional to organic management. In this study, decreased β-lactam resistance rate of CNS parallels the discontinuation of the use of β-lactam antibiotics. Aggregate results of this longitudinal study and other cohort studies, comparing cows managed conventionally vs. organically, suggest that the cessation of the use of antibiotics on a dairy farm transitioning to the organic management decreases pathogens that are resistant to antibiotics.

P2-155 *Lactobacillus helveticus* mRNA Expression Levels Related to Stress Response and Specific Metabolic Activity: Growth in Broth and throughout Swiss-type Cheese Manufacture and Ripening

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Introduction: Emmental cheese is a Swiss-type cheese with a complex microbiota composed of starter bacteria and non-starter lactic acid bacteria which have successive implications in cheese manufacture and ripening.

Purpose: The aim of this work was to compare *Lactobacillus helveticus* ITG LH56 expression levels associated with metabolic activity and stress response during growth (i) in MRS broth at 37°C or (ii) during cheese manufacture and ripening.

Methods: Population quantifications were performed by enumeration on specific agar media and quantitative PCR, while gene expression levels were quantified by RT-Q-PCR targeting 16S rRNA as well as tuf and groL mRNAs. All experiments were carried out in triplicate, i.e., 3 independent cultures and 3 semi-industrial pilot scale Emmental cheese productions made from raw microfiltered milk inoculated with *Lactobacillus helveticus* ITG LH56, *Streptococcus thermophilus* ITG ST88, *Propionibacterium freudenreichii* ITG P14 and *Lactobacillus paracasei* ITG LC225.

Results: During pure culture in broth, *L. helveticus* population reached 8.92 log with a growth rate of 0.994h-1. Growth was correlated with a high percentage of mRNA expression levels associated with *tuf* gene, while mid-exponential and stationary growth phases were associated to an increasing expression of *groL* which reached 50% expression level, in log transformed values. During cheese manufacture, *L. helveticus* reached a maximum of

population (growth rate of 0.847h-1) during the acidification process with an mRNA percentage expression levels comparable to the one observed in pure culture for mid-exponential and stationary growth phases. During cheese ripening in cold and warm room, culturable population is 1 log lower than Q-PCR quantifications, with an increasing percentage of expression related to stress response.

Significance: This study reports how recording of targeted mRNA expression levels along the cheese manufacturing and ripening process could enable correlations between bacterial physiology or metabolic activity of interest to better understand and improve online industrial fermentation processes.

P2-156 Development of a Testing Procedure to Select Strains of *Listeria monocytogenes* for Use in Cheese Challenge Studies

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Introduction: Challenge studies in which pathogens are introduced into a food must be realistic and rigorous so that conclusions have research and regulatory validity. The criteria used for strain selection are a primary concern. A review of research published since 1987 revealed that pH may critically affect growth of *Listeria monocytogenes* in a wide variety of cheeses, whereas the influence of percent water-phase salt (% WPS) on growth was not apparent.

Purpose: We developed a testing procedure for screening strains of *L. monocytogenes* for growth potential in cheese at 10°C. A model cheese system (Richard's broth) containing lactate, glycerol, casamino acids, and yeast extract was prepared at pH 5.0 containing 0% WPS and pH 5.6 or 6.5 containing 0, 4 or 8% WPS.

Methods: The pH of the medium was adjusted with concentrated NaOH after autoclaving and before filter sterilization. The prepared medium (9 mL/tube) was inoculated with one of ten *L. monocytogenes* cultures and incubated at 10°C for up to 120 h with samples taken throughout storage. *L. monocytogenes* strains chosen for screening were clinical or dairy-product isolates. At each sampling point, a 1.0 mL sample was withdrawn, serially diluted in Butterfield's phosphate buffer, and surviving cells enumerated on Brain Heart Infusion agar following incubation at 37°C for 48 h.

Results: Results indicated significant strain-to-strain variation ($P < 0.05$). None of the strains grew at pH 5.0/ 0% WPS, pH 5.6/ 8% WPS, or pH 6.5/ 8% WPS. Only six of the ten strains grew within 72 hours in Richard's broth with pH 5.6/ 4% WPS, while eight strains grew within 48 h in pH 6.5/ 4% WPS Richard's broth.

Significance: This screening method confirmed that pH is a critical factor affecting growth of *L. monocytogenes* at 10°C. Contrary to previous research, our findings suggest that % WPS is also a critical parameter. This model system will be useful in detecting strain-to-strain variation in tolerance to critical environmental parameters. Use of this screening technique will allow selection of stress-tolerant strains for future cheese challenge studies.

P2-157 Efficacy of 3M™ Petrifilm™ Aerobic Count Plates to Enumerate *Bacillus sporothermodurans* and *Geobacillus stearothermophilus* in Milk

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Introduction: Although heat procedures to reduce the microbial load or to sterilize milk or dairy products are employed, heat-resistant, sporeforming bacilli such as *Geobacillus stearothermophilus* and *Bacillus sporothermodurans* can grow and proliferate in products.

Purpose: In this study, the effectiveness of the 3M™ Petrifilm™ Aerobic Count [AC] Plate to enumerate *B. sporothermodurans* and *G. stearothermophilus* in ultra high temperature [UHT] milk was determined, and its efficacy compared with pour-plate agar and one-streak nutrient agar methods.

Methods: Spores of *B. sporothermodurans* and *G. stearothermophilus* were produced in media appropriate for each strain. Tubes with 9 mLs of UHT milk were inoculated with 1mL-spore suspensions of *B. sporothermodurans* or *G. stearothermophilus* to give different spore concentrations. Inoculated milk was heat shocked (80°C for 10 min), and incubated at 37°C and 55°C. Samples were withdrawn at 0, 24, 48, and 72 h of incubation and 100 µL inoculated onto 3M Petrifilm AC Plates, 100 µL inoculated onto pour plates, and 10µL one-streak plates.

Results: Bacillus sporothermodurans and *G. stearothermophilus* were detected by the three methods analyzed; however, in some cases, Petrifilm AC plates detected the microorganisms faster than the pour plate and the one-streak methods. *Bacillus sporothermodurans* was detected at 9 h on the Petrifilm AC plate, compared to 18 h for both the pour plate and one-streak methods. Depending on the strain, *G. stearothermophilus* was detected between 6 to 12 h by the Petrifilm AC plate method, and between 9 to 12 h by the pour plate and one-streak methods.

Significance: The Petrifilm AC plate method showed good reproducibility when compared with the traditional methods and is compatible with industry's drive for rapid methods.

P2-158 An Integrated Cell Culture-PCR Assay for the Detection of *Coxiella burnetii* Nine Mile Phase II RSA 439 in Fluid Dairy Products

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Introduction: Coxiella burnetii (*Cb*), an obligate intracellular bacterium, has been used as the reference organism for defining milk pasteurization conditions. With renewed interest in minimizing processing times and temperatures, and in ensuring product safety, development of an Integrated Cell Culture-PCR (ICC-PCR) assay may be useful for evaluating *Cb* inactivation in fluid dairy products.

Purpose: To develop the ICC-PCR assay, determine the detection limit, and characterize inhibiting effects contributed by various milk formulations.

Methods: Coxiella burnetii was inoculated on Vero cell culture with PBS and whole milk, incubated for 2 h to allow infection, and then incubated for 11 days to allow propagation. The propagated *Cb* mix was subjected to freeze-thaw followed by DNA extraction either by boiling the mix or spin column extraction. Extracted DNA was amplified using TaqMan-MGB based real-time PCR targeting published primers for the IS1111 transposase gene to verify *Cb* growth/infectivity and best extraction method. For detection limit determination, serial dilutions of *Cb* in RPMI were mixed separately in whole milk, cream, chocolate milk and eggnog. The mix was overlaid on sub-confluent Vero cell monolayers, subjected to freeze-thaw followed by spin column extraction and PCR. Uninoculated wells were evaluated for dairy sample background signal, and inhibition was evaluated by comparison to purified DNA. Duplicate trials using 6 replicates per sample were performed.

Results: After 11 days, infectivity of *Cb* in Vero cells was demonstrated by a decreased PCR cycle threshold equivalent to 2-3 log increase in genome equivalents (ge)/ml. Spin column extraction was superior to boiling for DNA extraction. ICC-PCR inhibition varied, with chocolate milk > eggnog > cream > whole milk. Detection limit was 50 *Cb* (ge)/well for whole milk and cream, and 500 (ge)/well for chocolate milk and eggnog. Background signals were negligible.

Significance: This ICC-PCR assay detects infectious *Cb*, and may be used to study factors affecting *Cb* inactivation via pasteurization.

P2-159 Reduction of *Salmonella* on Jalapeño Peppers, Peanuts and Dry Dog Food Utilizing Targeted Directional Microwave Technology

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Introduction: *Salmonella* contamination of jalapeño peppers, shelled peanuts, and dog food continues to be a concern. These concerns provides evidence of the need for post harvest interventions.

Purpose: The objective of this experiment was to assess the microbial reduction of *Salmonella* on jalapeño peppers, dry dog food and shelled raw peanuts by various doses of targeted directional microwaves (TDM).

Methods: Fresh jalapeño peppers, dried commercial dog food, and shelled peanuts were inoculated with a three strain *Salmonella* inoculum to achieve 10^5 CFU/gram. Separately, twenty-five gram samples of each product in duplicate were treated with directional microwave treatments for 0, 10, 20, 30 or 40 seconds for jalapeño peppers and 1, 2, 3, and 4 minutes for the dog food and peanuts. Serial dilutions, plating on XLD agar with TSA overlay, and incubation at 37°C occurred after treatment. Typical colonies were counted and total surviving *Salmonella* was determined. Experiment was replicated five times.

Results: Jalapeño peppers treated with TDM while tumbling can significantly reduce *Salmonella* by as much as 1.1 log CFU/g after 40 seconds ($P = 0.0028$). After 10 seconds 0.68 log CFU/g reduction occurred, after 20 seconds 1.08 log CFU/g, and after 30 seconds 0.92 log CFU/g reduction occurred ($P < 0.05$). *Salmonella* on the dog food was reduced by 1.49 log CFU/g after 1 minute, 1.75 log CFU/g after 2 minutes 1.08 log CFU/g, 2.12 log CFU/g after 3 minutes and 2.6 log CFU/g after 4 minutes ($P < 0.05$). *Salmonella* on the shelled peanuts was reduced by 0.50 log CFU/g after 1 minute, 1.15 log CFU/g after 2 minutes, 1.56 log CFU/g after 3 minutes and 2.2 log CFU/g after 4 minutes ($P < 0.05$).

Significance: TDM technology has been shown through this experiment to provide 1–2 log CFU/g reduction in *Salmonella* in a short amount of time.

P2-160 Inactivation of *Escherichia coli* O157:H7 by Copper Alone or in Combination with Lactic Acid as Demonstrated by Scanning Electron Microscopy

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Introduction: *E. coli* O157:H7 is a foodborne pathogen of considerable public health and food safety importance. Copper and lactic acid have been shown to have an antimicrobial effect against *E. coli* O157:H7. Copper in combination with lactic acid is a promising non-thermal method to reduce the risk of foodborne pathogens.

Purpose: The objectives of this investigation were (a) to study efficacy of copper alone or in combination with lactic acid against *E. coli* O157:H7 in laboratory medium, and (b) to determine the effect of copper and lactic acid treatment on the cell morphology using scanning electron microscopy.

Methods: Brain Heart Infusion Broth (BHI) was prepared with 0, 5, 10, 20, and 40 ppm copper alone or in combination with 0.1 and 0.2% lactic acid. Samples were inoculated with *E. coli* O157:H7 at approximately 3 log CFU/ml. The inoculated samples were incubated at 37°C for 8 hr. At the end of the incubation period, samples were analyzed for bacterial population. Scanning electron microscopy was also used to observe bacterial morphology.

Results: Addition of 40 ppm copper to 0.2% lactic acid reduced the population of *E. coli* O157:H7 by 5 log CFU (recommended by FDA) when compared to the control. The bacterial cell morphology indicated that copper and lactic acid altered the size and shape of *E. coli* O157:H7, thus inhibiting the growth of microbial cells. Therefore, the results suggest that copper and lactic acid act synergistically ($P < 0.05$) to inhibit the growth of *E. coli* O157:H7.

Significance: Based on our studies, copper in combination with lactic acid have potential for controlling growth of *E. coli* O157:H7. This could be a novel and encouraging alternative approach to be used in food systems.

P2-161 Antimicrobial Effect of Three Lactic Acid Bacteria against *Listeria monocytogenes*

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Introduction: Control of *Listeria monocytogenes* in ready-to-eat (RTE) food products is a significant challenge, and improved means for its control are needed.

Purpose: In this study, the anti-listerial effectiveness of three Lactic Acid Bacteria (LAB) was investigated.

Methods: Spot-on-lawn and minimum inhibitory concentration (MIC) assays were performed to assess the anti-listerial effects of the three LAB.

Results: Spot-on-lawn assays conducted at 4, 7, 23, and 37°C clearly demonstrated the effectiveness of one of the three strains (*Pediococcus acidilactici*). Zones of *L. monocytogenes* inhibition by *P. acidilactici* ranged from 15 to 18mm (23 and 4°C, respectively). A study of the effect of *P. acidilactici* growth phase on its anti-listerial effectiveness determined that effectiveness was greater during stationary phase. After only 8h of growth (or late log phase), the zone of inhibition was 8 mm, the same size as the spot itself. However, after 48h of growth (or late stationary phase), the zone of inhibition grew to 14 mm. Interestingly, it was observed that the zones of inhibition for the 4 and 7°C spot-on-lawn assays increased over time, perhaps due to increased time for diffusion of the antimicrobial or an increase in its efficacy against the slowly growing *L. monocytogenes*. Furthermore, it was observed that the tendency for *L. monocytogenes* to develop spontaneous resistance was reduced during exposure to *P. acidilactici* at refrigeration temperature. Additional, spot-on-lawn assays using *P. acidilactici* cell free supernatant (CFS) supported the preliminary results and confirmed the effectiveness of *P. acidilactici*. MIC experiments using CFS from the three strains revealed that while two of the LAB were minimally effective at inhibiting *L. monocytogenes*, *P. acidilactici* was able to inhibit its growth up to a 1:256 dilution after 24 h of incubation at 23°C.

Significance: These data demonstrate that *P. acidilactici* may represent an effective control for *L. monocytogenes* in RTE products using *P. acidilactici* in their formulations.

P2-162 Host Spectrum of *Escherichia coli* Specific Phages Isolated from Hog Fecal Samples

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Introduction: According to the World Health Organization, 5 million children die every year due to severe diarrhea. *Echerichia coli* is one of the most responsible organisms involved in acute diarrhea worldwide. Based on a report from the Korea Food and Drug Administration, pathogenic *E. coli* has been the biggest problem in foodborne human illness in Korea, and most isolates were resistant to penicillin (63.4%) and/or tetracycline (42.8%).

Purpose: In this study, we isolated *E. coli* specific bacteriophages from hog fecal samples and applied them to 100 *E. coli* isolates from hog farming environments, foods, and clinical samples in a same geographical location.

Methods: For the isolation of phages, samples were collected from a hog-waste treatment plant in Gyeonggi-do, Korea, and *E. coli* NCCP 13721 was selected as an indicator of phage presence. Susceptibility of the strains against phage infection was determined by using dotting or double-layered plaque assay. Pathogenicity and antimicrobial-resistance were also investigated in the *E. coli* isolates other than phage susceptibility.

Results: From the hog fecal samples, six isolated coliphages were applied to *E. coli* isolates. Of the 100 *E. coli* strains, 44% of food isolates and 50% of the isolates from hog farming environments were susceptible to the phage infection. Interestingly, 85% of the isolates from human fecal samples were barely grown in the presence of phages. The phages were more effective in control of the isolates which were involved in human illness. We failed in determination of any relation between antimicrobial resistance and phage susceptibility in the isolates. Phages could control *E. coli* isolates regardless of drug-resistance profile.

Significance: The findings in this study suggest that coliphages may be a useful tool to suppress the level of *E. coli* contamination in foods and hog farming environments, potentially resulting in lowering human illness.

P2-163 High Level Antimicrobial Resistance in *Escherichia coli* from Foods, Poultry and Humans in the Northern Region of Ghana

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Introduction: Antimicrobial resistance surveillance and monitoring is one of the major tools used in the developed world to track down the flux of resistance genes in bacteria. Surveillance and monitoring are virtually unavailable couple with the irrational use and poor quality of antibiotics used in animal production and individuals in developing countries.

Purpose: We performed this research to determine the levels of resistance of *Escherichia coli* isolated from foods, poultry and patients from Tamale, located in the poorest area in the country, the Northern Region.

Methods: Standard microbiological guidelines were used to isolate and identify *E. coli* phenotypically, biochemically and molecularly. PFGE was performed in all isolates. One hundred and one (101) human fecal samples, 49 street food samples, and 30 poultry fecal samples were taken for this research. Antibiotic susceptibility test was performed following the guidelines and breakpoints of the European Union Committee on Antimicrobial Susceptibility Testing, EUCAST.

Results: We isolated 15 *E. coli* from food, 99 of the human fecal samples and 30 from all the poultry fecal samples. There were high levels of resistance to the commonly used antibiotics in this area especially from the human and poultry samples. Eighty percent (80%) of the poultry isolates exhibited multi-drug resistance while 59% of those from human origin recorded multi-drug resistance. Surprisingly, 40% of the isolates from Street foods also exhibited multi-drug resistance to the 20 antibiotics tested. There was resistance to amoxicillin-clavulanic in both food and human samples as well resistance to cefotaxime and ceftazidime in the human isolates.

Significance: Food is involved involved in transmission of antibiotic resistant *E. coli* in Ghana. Our data suggest that these bacteria originate from the human manipulators by cross-contamination, rather than from animals. Thus, education in Ghana is crucial to prevent further fecal contamination of Street Food. Further, there is emergence of resistance of *E. coli* to third generation cephalosporin antibiotics that are not commonly used in the area and amoxicillin-clavulanic that serves as the antibiotic of last resort in Ghana.

P3-01 Performances Assessment of the CampyFood Agar Method According to the ISO 16140 Standard in Poultry and Meat Products, in Environmental Samples

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Introduction: CampyFood Agar (CFA, bioMérieux) is a chromogenic medium for enumeration and presumptive identification of *Campylobacter* spp. in poultry, meat and environmental samples. *Campylobacter* spp. strains grow producing typical red colonies. It is up to users to select the primary diluent or the confirmatory test that is the most appropriate to their routine analyses.

Purpose: A study was conducted at ADRIA to validate this new method in comparison to the ISO/TS 10272-2 reference method, as part of the Microval approval process, and according to the ISO 16140 standard.

Methods: Samples diluted in Buffered Peptone Water or in peptone salt as recommended in ISO standards were inoculated onto the CFA spread plates. Primary dilution in the selective CampyFood broth was also tested. The plates were incubated for 48 hours at 41.5 ± 1°C. Characteristics colonies were further confirmed using the ISO/TS 10272-2 confirmatory tests, using a simplified cultural procedure, or using the VIDAS CAM assay performed on a pool of 5 colonies.

Results: Whatever the diluent used for primary dilution and the confirmation methods, the CampyFood method showed satisfying relative linearity performances, with linear correlation coefficients superior to 0.95 and approved lack-of-fit tests. Biases between both methods were characterized by low values, varying from -0.15 to + 0.13 log CFU/g. The intercepts close to 0 and the slopes close to 1 were validated for all the tested categories in the accuracy study. The selectivity and specificity of the CampyFood agar method were shown to be good by testing 41 target and 28 non target strains. The limits of repeatability and reproducibility of the CampyFood Agar method, which were calculated during an inter-laboratory study involving 13 labs, were similar to those of the ISO/TS 10272-2 standard method. As already observed in the methods comparison study, biases between the methods were clearly non significant, varying from -0.01 to -0.14 log CFU/g.

Significance: According to this performance assessment study, the CampyFood Agar method was certified as an alternative method to the reference ISO/TS 10272-1 reference method for enumeration of *Campylobacter* spp. in poultry, meat and environmental samples. It represents a valuable and user friendly alternative method, by providing final results in two days when using the VIDAS CAM assay as confirmatory test.

P3-02 Performances Assessment of the TEMPO STA® Method According to the ISO 16140 Standard for Coagulase-positive Staphylococci Enumeration

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Introduction: TEMPO® system (bioMérieux) is an automated method associating an innovative card with a medium adapted to ensure a rapid enumeration of several quality indicators. It replaces serial dilutions and tedious plate reading with a simple 1:10 dilution and an automated enumeration based on a miniaturized card using 16 tubes MPN (Most Probable Number) method. The TEMPO® STA method allows the coagulase-positive *staphylococci* enumeration in foods and pet foods.

Purpose: A study was conducted to validate the TEMPO® STA method in comparison to the ISO 6888-2 standard, as part of the AFNOR Certification approval process and according to the ISO 16140 standard.

Methods: Samples were analyzed by the compared methods, i.e., the TEMPO® STA and the ISO 6888-2 method.

Results: The TEMPO® STA method showed satisfying relative linearity performances, with linear correlation coefficients superior to 0.98. Biases between both methods were characterized by low values, varying from -0.00 to +0.18 log CFU/g. The intercepts close to 0 and the slopes close to 1 were validated for all the tested categories in the accuracy study. The selectivity and specificity of the TEMPO® STA method were verified by testing 31 target and 24 non-target strains. The limits of repeatability and reproducibility of the TEMPO® STA method, which were calculated during an inter-laboratory study involving 16 labs, were similar to those of the 6888-2 standard method. Biases between the compared methods were clearly non significant, varying from -0.00 to -0.26 log CFU/g. At least, TEMPO® system offers important economic savings by standardizing the analysis, as well as minimizing the volume of wastes, the training and handling times.

Significance: According to these performances assessments, the TEMPO® STA method represents a valuable and user friendly alternative method for coagulase-positive staphylococci enumeration in food and pet food stuffs.

P3-03 Accelerated Procedures for Detection and Isolation of *Listeria* spp. from Food Samples

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Introduction: Listeria monocytogenes is a significant foodborne pathogen that is widely distributed in nature and can be found in a wide range of foods including milk, vegetables, meat and processed foods.

Purpose: Rapid screening techniques including the VIDAS LIS and VIDAS LMOII and Neogen and SDIX lateral flow devices were evaluated for detection of *Listeria* spp. and *Listeria monocytogenes* in artificially contaminated foods.

Methods: Food matrices tested included: asadero cheese, queso fresco, brie cheese, guacamole, coleslaw and smoked salmon. Inoculation levels used were ~ 0.1 CFU/g, ~1.0 CFU/g and ~10 CFU/g. Six replicates at each inoculum level were tested per food matrix, as well as six uninoculated controls. Two enrichment procedures were used, Buffered *Listeria* Enrichment Broth (BLEB) which is used with the VIDAS LIS test and Demi Fraser (DF) broth which is subcultured into and Fraser Broth (FB) and used with the VIDAS LMO II assay. Enrichment broths were also streaked onto Oxford agar and R&F *Listeria monocytogenes* Chromogenic Plating Medium for cultural isolation. Confirmation of isolates to the species level was accomplished by purifying isolates on TSAYE agar and performing a Gram stain, catalase test, hemolysis test, motility test, and real time PCR. A real-time 5'-nuclease PCR assay targeting regions of the *iap* gene to simultaneously detect *L. monocytogenes* as well as all *Listeria* species was used for confirmation of isolates.

Results: When samples artificially contaminated with *Listeria monocytogenes* were incubated in BLEB for 24 h at 30°C, the RapidCheck and Neogen devices detected the organism in 39.8% and 67.59% of samples, respectively, while those enriched in DF broth showed 33.3% by the RapidCheck and 52.8% by the Neogen devices. Cultural isolation from the 24-h enrichments were 87.0% for BLEB and 97.2% for DF. Both the RapidChek and Neogen Reveal tests worked as well as the VIDAS-LIS and VIDAS-LMOII methods and cultural isolation on selective agar after 48h enrichment.

Significance: Both the RapidChek and Neogen Reveal devices can be used as alternatives to the VIDAS screening assays for detection of *Listeria* spp. in 48 h enrichment broths. Cultural isolation was reliable for detection of *Listeria* spp. after 24-h enrichment.

P3-04 A Comparative Evaluation of Petrifilm™ Aqua Plate Methods vs. Various Reference Methods in Testing of Bottled Water

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Introduction: The Petrifilm™ Aqua Heterotrophic Count(AQHC) Plate, Aqua Coliform Count (AQCC) Plate, Aqua *Enterobacteriaceae* Count Plate(AQEB)and Aqua Yeast and Mold (AQYM) plates have been developed to facilitate enumeration of the specified indicator organisms for testing bottled water.

Purpose: To compare the counts from bottled waters using both the Petrifilm plate methods and the reference/customer methods.

Methods: Two lots each of 15 different matrices, representing 4 types of water: purified municipal, regional spring, purified and natural spring waters were tested. Each lot was spiked with organisms typically isolated from water wherever possible. For each matrix and organism, 3 spike levels were tested: zero, low/medium, and medium/high- by Petrifilm Aqua Plates and reference/customer methods in duplicate. The Petrifilm AQHC plate was compared to the SMEWW & ISO methods for heterotrophic bacteria through direct plating and membrane filtration. The Petrifilm AQCC plate was compared to SMEWW & ISO methods for coliform, through membrane filtration. The Petrifilm AQEB plate was compared to a customer method for *Enterobacteriaceae* through membrane filtration. The Petrifilm AQYM method was compared to the SMEWW and a customer method for yeast & mold through membrane filtration.

Results: Analysis of variance showed that there was no statistical difference between the Petrifilm Aqua Plate methods and their respective reference methods (*P*-value=0.727 and 0.739 for Petrifilm AQHC plate compared to ISO method at 22°C and 36°C using direct plating and *P*-value=0.870 compared to SMEWW method with membrane filtration; *P*-value=0.38 and 0.08 for the Petrifilm AQCC plate compared to the SMEWW and ISO using membrane filtration, respectively; *P*-value=0.44 for the Petrifilm AQEB plate compared to a customer method using membrane filtration; *P*-value=0.99 and 0.11 for the Petrifilm AQYM plate compared to a customer method with membrane filtration at 25°C for 3 and 5 days respectively and *P*-value=0.19 compared to SMEWW with membrane filtration at 20°C for 3 days).

Significance: All Petrifilm™ Aqua Plate methods have demonstrated comparable results to the ISO and SMEWW reference and customer methods. The use of a ready-made systems allows for a more streamlined and standardized process for analysis of bottled water.

P3-05 Evaluation of a New Phage Ligand Assay for the Detection of *Listeria* Species in Foods and Environmental Samples

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Introduction: Rapid screening for *Listeria* is important in food safety because of the potential to prevent serious disease and the ability to release product sooner.

Purpose: The objective of this study was to demonstrate the effectiveness of a new phage ligand assay (VIDAS LPT) method for rapid screening of *Listeria* species in a variety of food products and environmental samples when compared to the traditional ISO 11290-1 method.

Methods: In the new method, samples are enriched at 30°C in a proprietary ready to use broth for 24-26 hours (surfaces) or 26–30 hours (foods) prior to testing on the automated instrument. All positive samples were confirmed by plating onto a selective *Listeria* agar plate. The new test incorporates a recombinant phage protein to increase the sensitivity and specificity compared to traditional immunoassays.

Results: In this study, 502 food samples representing a wide variety of foods and 200 environmental samples were tested. Forty percent of the products were naturally contaminated. Three hundred four products tested positive by one of the methods and the sensitivity was found to be 86.8% for the alternative method and 86.3% for the ISO 11290-1 method. MacNemar's analysis at the 5% level showed no significant difference between the VIDAS LPT and the reference method.

Significance: The alternative method provides the detection of *Listeria* species in foods with negative or presumptive positive results in less than 28 hours compared to at least 5 days for the cultural methods. This simple method, with one single enrichment, also significantly decreases the workload of the laboratories.

P3-06 ISO 16140 Validation Study of the ALOA® One Day Method for *Listeria* Species Detection in Foodstuffs and Environmental Samples

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Introduction: ALOA® One Day is a certified method for *Listeria monocytogenes* detection. It has recently been granted validation for the detection of *Listeria* species in foodstuffs and environmental samples. The proposed protocol is simpler, shorter and cheaper than the ISO mandatory reference method.

Purpose: The aim of this study was to compare the ALOA® One Day alternative method to the NF EN ISO 11290-1 standard according to the ISO 16140: 2003 requirements. Relative specificity, relative accuracy and limit of detection were tested for both methods.

Methods: The specificity of both methods (ALOA® One Day and NF EN ISO11290-1) were compared by testing 63 *Listeria* species (inclusivity) and 32 non-Listeria species (exclusivity). Three hundred seventy-nine samples including 123 naturally-contaminated samples were tested by the reference and the alternative method. During these tests relative accuracy, sensitivity and specificity were calculated. ALOA® One Day is a 2-step method: an enrichment step (24 h at 30°C in half-fraser) and a detection step (subculture of 0.1 ml on one ALOA® plate and incubate 24h at 37°C). The positive samples must be confirmed by a 5-h procedure using one typical colony and a lateral flow test.

Results: Exclusivity and inclusivity data have shown a perfect recovery of all the *Listeria* species and a perfect discrimination or inhibition of the 30 non-*Listeria* species strains. The statistical analysis performed on the 379 food samples tested according to ALOA® One Day and ISO 11290-1 has proved that the two methods were equivalent: relative accuracy (AC) = 99.4 %, relative specificity (SP) = 99.45 % and relative sensitivity (SE)=99.49 %.

Significance: ALOA® One Day is a suitable method to detect *Listeria* species in foodstuffs, animal feeding and environmental samples. This method showed equivalent specificity and sensitivity to the ISO reference method and enables laboratories to save time and money.

P3-07 Dipstick Assay for *Vibrio vulnificus*

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Introduction: Vibrio vulnificus is considered to be the most invasive *Vibrio* in the U.S causing high fatalities, especially in immunocompromised individuals. The rapid detection of *V. vulnificus* is necessary for monitoring this pathogen in the environment and in seafood as a pro-active measure to reduce *V. vulnificus*-related infections.

Purpose: The purpose of this study was to develop a rapid, user- friendly and compact screening dipstick device utilizing *V. vulnificus* anti-H monoclonal antibodies, which can detect the presence of *V. vulnificus* from oyster homogenate within 5 min.

Methods: The dipstick test strips were prepared by conjugating a species specific anti-*V. vulnificus* H monoclonal antibody, and colloidal gold particles. The resultant antibody conjugate was dispensed onto a membrane (15 µ pore size) at a rate of 1 µl/cm. A control line was prepared by using goat anti-mouse IgG and was sprayed on the membrane at a rate of 1.5 µl/cm. Specificity of the dipstick device was tested against pure cultures of 8 *Vibrio* strains, while sensitivity was tested by utilizing serially diluted overnight grown *V. vulnificus* culture (10^6 to 10^1 CFU/ml). Sensitivity of the device was also tested with oyster meat homogenate (10g oyster meat + 20 ml APW), spiked with *V. vulnificus* ATCC 27562 to reach concentrations from 10^6 to 10^1 CFU/ml. The samples were incubated at 35°C and 1 ml from each dilution was collected every hr for 6 hrs and immediately tested with the dipstick.

Results: The dipstick device successfully identified *V. vulnificus* and did not produce visible signals for other *Vibrio* strains tested within 5 min. The lowest concentration of *V. vulnificus* that produced positive test strip results was 10^4 CFU/ml. When combined with a 5 hr enrichment period, sensitivity of the dipstick increased to 10^1 CFU/ml.

Significance: Our dipstick assay could serve seafood industries for rapid pathogen detection.

P3-08 Reveal *Salmonella* Enteritidis Test for Rapid Detection of *Salmonella* Enteritidis in Shell Eggs and Environmental Samples

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Introduction: Ingestion of *Salmonella enterica* serovar Enteritidis (SE) contaminated eggs is one of the major causes of *Salmonella* associated gastroenteritis in humans. Thus, SE monitoring in eggs and poultry layer house environments is critical for ensuring food safety. Reveal *Salmonella* Enteritidis (Reveal SE) is a lateral flow-based immunodiagnostic assay, which employs highly specific antibodies to accurately detect SE.

Purpose: In this study, Reveal SE was evaluated for the rapid detection of SE from pooled shell eggs and environmental samples.

Methods: Low levels of SE were inoculated into pooled shell eggs and enriched according to the procedure prescribed in the Bacteriological Analytical Manual (BAM), U.S. Food and Drug Administration (FDA). Uninoculated samples were included in each trial. As a part of the enrichment procedure, samples were held at room temperature for 96 h prior to pre-enrichment. A new abbreviated 48 h/ no hold enrichment procedure was also tested for pooled shell egg sample testing. Studies included screening of 245 natural drag swabs and inoculation trials with drag swabs. Enrichment was done per the procedure outlined in the BAM method. In addition to internal studies, independent laboratory trials were conducted with both pooled shell eggs and poultry house environmental samples.

Results: In both internal and independent laboratory evaluation of pooled shell eggs, Reveal SE exhibited 100% sensitivity and 100% specificity in comparison to the reference method. Chi-square analysis revealed that the performance of abbreviated 48 h/ no hold enrichment procedure was statistically equivalent to that of the reference procedure. Natural drag swab screening of environmental samples produced three Reveal SE positive samples, which were confirmed by the reference procedure, resulting in 100% sensitivity and 100% specificity. External laboratory testing of 147 poultry house samples resulted in 35 Reveal SE confirmed positives, resulting in sensitivity of 100% and specificity of 90%. Inoculation trials with drag swabs resulted in 96% sensitivity and 100% specificity.

Significance: Results obtained demonstrate that Reveal SE is a highly sensitive and specific assay for rapid detection of SE from both pooled shell eggs and environmental samples. Also, for pooled shell egg sample testing, the abbreviated/ no hold enrichment procedure can be used in conjunction with the Reveal SE test, achieving a significant enrichment time savings of 96 h.

P3-09 Development of Colloidal Gold-based Lateral-flow Immunoassay for the Rapid Simultaneous Detection of *Listeria monocytogenes* and *Escherichia coli* O157:H7

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Introduction: Every year, millions of people in the world suffer from foodborne disease, and foodborne pathogens continue to be a major public health concern. Among the known foodborne pathogens, *Escherichia coli* O157:H7 and *Listeria monocytogenes* are of major concern because of their continued association with highly popular foods such as poultry products, ready-to-eat meats, dairy products, and fruits and vegetables. These pathogens are emerging high-risk food pathogens that have been involved in a number of recent outbreaks. Therefore, the control and prevention of these pathogens are of high priority to improve the safety of the food supply. The accurate and rapid detection of these pathogens is essential.

Purpose: The purpose of this study was to develop a colloidal gold-based lateral-flow immunoassay for the rapid, simultaneous detection of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in food samples.

Methods: Heat-killed cells of *Listeria monocytogenes* ATCC 19113 and *Escherichia coli* O157:H7 ATCC 43888 were used as immunogens to immunize mice, respectively. Monoclonal antibodies were developed using B-cell hybridoma technology. Colloidal gold was prepared by the sodium citrate reduction method and conjugated to monoclonal antibodies specific to *Listeria monocytogenes* and *Escherichia coli* O157:H7, respectively. With the colloidal gold-MAb conjugates, one step of lateral-flow immunoassay was developed for rapid, simultaneous detection of *Listeria monocytogenes* and *Escherichia coli* O157:H7.

Results: Monoclonal antibodies specific to *Escherichia coli* O157:H7 (EC2b5 Mab) and *Listeria monocytogenes* (LM5E7 Mab) were produced from cloned hybridoma cells. The nitrocellulose membrane of the lateral-flow immunoassay was treated with Mab-EC2b5, Mab-LM5E7 and anti-mouse IgG in the *Escherichia coli* O157:H7 test, *Listeria monocytogenes* test and control zones, respectively. Monoclonal antibody-gold conjugates(EC2b5 Mab-gold and LM5E7 Mab-gold) were sprayed onto the conjugate pad. The detection limits of the lateral-flow immunoassay were 10^4cell/ml and 10^5cell/ml for *Escherichia coli* O157:H7 and *Listeria monocytogenes*, respectively, and the results were obtained within 15 min after starting the analysis.

Significance: Multipathogen detection on a single-assay not only reduces the cost for testing but also provides data on the presence of pathogens in a single experiment.

P3-10 Development of IC-ELISA for Detection of Mycotoxin Patulin in Food Samples

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Introduction: Patulin is a toxic secondary metabolite of a number of fungal species belonging to the genera *Penicillium* and *Aspergillus*. It has been mainly isolated from apples and apple products, but it has also been extracted from rotten fruits, moldy feeds and stored cheese. Human exposure to mycotoxins occurs by ingestion of contaminated products and can lead to serious health problems, including immunosuppression and carcinogenesis. The content of patulin in foods has been restricted to 50 ppb in many countries. Conventional analytical detection methods involve chromatographic analyses, such as HPLC, GC, and, more recently, techniques such as LC/MS and GC/MS. However, extensive protocols of sample cleanup are required prior to the analysis, and to accomplish it, expensive analytical instrumentation is necessary. An immunochemical analytical method, based on highly specific antigen-antibody interactions, would be desirable, offering several advantages compared to conventional techniques, i.e., low cost per sample, high selectivity, high sensitivity and high throughput.

Purpose: Development of IC-ELISA for rapid detection of patulin in food samples.

Methods: In this study, two haptens of patulin were synthesized. By the active esters method, the haptens were conjugated to bovine serum albumin to be used as the immunogen for the production of monoclonal antibodies, and conjugated to ovalbumin to be used as coating antigen. By using the monoclonal antibody and the coating antigen, an IC-ELISA has been developed and used to detect patulin in food samples.

Results: Monoclonal antibodies specific to patulin were produced from cloned hybridoma cells. Then the monoclonal antibody-based indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) was developed and optimized. Under the established optimized conditions, the IC-ELISA showed an IC50 of 10 ng/mL with a detection limit of 0.09 ng/mL. The IC-ELISA showed negligible cross-reactivity with other mycotoxins. Recoveries of patulin from spiked apple juice samples ranged from 85 to 98%.

Significance: Monoclonal antibodies specific to patulin were developed in this study, and indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) was developed. The assay allowed the detection of 0.09 ng/mL patulin and thus represents a promising basis for the development of an alternative methodology compared to the analytical chromatographic techniques in use.

P3-11 Development of a Non-O157:H7 Enterohemorrhagic *Escherichia coli* Lateral Flow Device

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Introduction: Six strains of non-O157:H7 enterohemorrhagic *Escherichia coli* (EHEC) have been identified as a serious threat to food safety. In order to address the risk posed by these bacteria, antibody-based rapid tests are needed that can detect and identify these virulent strains.

Purpose: To develop an antibody-based lateral flow device to simultaneously detect six strains of non-O157 EHEC (O26, O45, O103, O111, O121, and O145).

Methods: High-titer polyclonal antibodies to each of the six EHEC strains (O26, O45, O103, O111, O121, and O145) were produced in goats by injecting the animals with heat-killed bacterial cells. Using affinity-purified goat IgGs, a prototype lateral flow device was developed with antibody-coated nanogold as the detection reagent. The device was tested against a panel of purified bacterial cells as well as spiked meat enrichment samples.

Results: The prototype EHEC lateral flow device detected each of the six EHEC strains when tested at 10^5–10^7 CFU/ml. The device also detected five of the six strains in 12-hour meat enrichments spiked with 2–14 CFU/375 g of meat. Each rapid test was able to correctly identify and distinguish its target strain from the other five, as well as from other bacteria.

Significance: The EHEC lateral flow device can be a valuable tool in efforts to simultaneously screen and detect six strains of non-O157:H7 enterohemorrhagic *Escherichia coli* (EHEC) from food samples.

P3-12 A New Automated Immunoassay Method for *Salmonella* Testing in Less Than Twenty Hours: Food Comparison Study

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Introduction: Salmonella testing is time consuming and the food industry requires rapid and simple methods for faster release of perishable foods and to decrease the laboratory workloads.

Purpose: The objective of this study was to evaluate the performances of a new automated next-day method for the detection of *Salmonella* in food, animal feed and environmental samples.

Methods: Samples are enriched in buffered peptone water, supplemented with a mix of selective agents for 18–24 h at 41.5 °C, prior to the detection on the VIDAS instrument. The detection test incorporates recombinant phage proteins associated to monoclonal antibodies to target specific somatic (O) and flagellar (H) *Salmonella* antigens, enabling the detection of both motile and non-motile strains. Positive results were confirmed by streaking the enrichment broth onto selective agar plates. This study compared the new method to the ISO 6579 reference method, according to the ISO 16140 standard requirements.

Results: The comparative study was performed on 334 products distributed in seven categories : meat, dairy, miscellaneous products, seafood & vegetables, raw milk cheeses and feed and environmental samples. Thirty eight per cent of positive samples were naturally contaminated and 62% were artificially inoculated with stressed *Salmonella* at low levels. One hundred eight samples were confirmed positive by one of the methods, 1 by the cultural method only and 107 by both methods. There was no significant difference between the alternative method and the reference method using the Mac Nemar's analysis at the 5% level.

Significance: The VIDAS UP *Salmonella* provided comparable results to the ISO 6579 reference method for the detection of *Salmonella* in food, animal feed and environmental samples, allowing release of negative products in less than 20 hours with the use of a unique simple enrichment followed by an automated detection.

P3-13 24-hour Test System for the Detection of *Salmonella* spp. on Environmental Surfaces

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Introduction: Salmonella causes 1 million cases of food poisoning and almost 400 deaths annually in the United States. Investigations into salmonellosis cases caused by processed foods across 43 states found that the pathogen generally entered the food supply through cross-contamination from the processing environment. Recurring outbreaks underscore the difficulty of eradicating *Salmonella* from the processing environment, highlighting the need to reinforce preventive control measures and routine monitoring of the processing environment for the presence of this pathogen.

Purpose: The aim of the study is to evaluate the performance of the RapidChek® SELECT™ *Salmonella* test system against the FDA reference method (BAM Chapter 5 *Salmonella*) for the detection of *Salmonella* spp. on environmental surfaces.

Methods: Method comparison studies were conducted on stainless steel, plastic, painted concrete, and rubber to determine accuracy, specificity, and sensitivity. One hundred twenty-five (125) samples were analyzed by both methods.

Results: The RapidChek® SELECT™ *Salmonella* test system demonstrated equivalent performance with regard to sensitivity, specificity, and accuracy as compared to the FDA reference method. The RapidChek method reported sixty-two (62) confirmed positive results, while the reference method reported fifty-nine (59) confirmed positive results. The overall accuracy of the RapidChek method was 105%, with an average sensitivity and average specificity of 100%.

Significance: Salmonella spp. can be detected at very low levels of contamination in the processing environment in as few as twenty-four (24) hours with the RapidChek method as compared to seventy-two (72) hours with the reference method which helps food producers quickly identify *Salmonella* in the environment before it contaminates finished food products, reducing disruption in production schedules and product hold times.

P3-14 Biosensor for Functional and Rapid Screening of Selected Pathogens and Toxins from Food

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Introduction: Rapid and discriminatory detection of viable pathogens or active toxins from non-viable cells or inactive toxins is important to prevent food related out-breaks and product recalls. Therefore, the need to develop a rapid sensor platform enabling functional elimination of "false-positives" resulting from non-viable cells or inactive toxins is highly desirable.

Purpose: The purpose of this study was to develop a biosensor-based rapid screening assay for rapid detection and discrimination of viable versus non-viable cells of pathogenic *Listeria*, the active versus inactivated toxin listeriolysin O (LLO) and the enterotoxin from *Bacillus* species from model foods and beverages.

Methods: Mammalian cells of mouse and human origin were encapsulated in alginate-collagen microspheres and arrayed onto chambered slides or in multi-well plates to construct the biosensing platform. Model food samples (hotdogs, fried rice, pasta, pasteurized milk, apple juice and packaged water) were spiked with crude toxin preparations (0.1 ng/ml-20 µg/ml) or bacterial cells (10^2–10^4 CFU/g). The samples were heated at different time-temperature combinations and were introduced to the biosensor. The cytotoxicity values of encapsulated biosensor cells were determined at 1–4 h post-infection by assaying intracellular enzyme release or by evaluating live/dead cells using fluorescence microscopy and image analysis.

Results: Non-heat treated food samples containing pathogenic *L. monocytogenes* strains or LLO preparations showed cytotoxicity ranging from 41% to 98%. The crude toxin preparation from pathogenic *B. cereus* strains showed cytotoxicity values of 30% to 100% (not heated). On the contrary, all heat treated food/beverage samples yielded significantly ($P < 0.01$) low cytotoxicity values (less than 10%), which were comparable to negative controls, such as non-pathogenic *L. innocua* (F4247) and *B. subtilis* induced cytotoxicity values of 2% and 7%, respectively. Live/dead status of the infected biosensing cells obtained by fluorescence microscopic image analysis also yielded similar cytotoxicity results.

Significance: This cell-based biosensor technology will bridge the *performance gap* in existing rapid microbiological laboratory methods by providing detection capabilities which are not only "rapid" but also "functional," in that it can distinguish between viable and non-viable pathogens or active and inactive toxins.

P3-15 Rapid and Specific Detection of STEC Strains O157:H7, O26 and O111 Using a Label-free Biosensor System

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Introduction: Since 1990, there have been at least 13 outbreaks of non-O157:H7 *E. coli* strains. Pending USDA testing guidelines for non-O157:H7 *E. coli* strains have raised the interests of several groups within the food production/protection communities. Classic immunoassays for foodborne pathogens rely on time-consuming enrichment steps, which may add several hours or days to these assays.

Purpose: The goal of this research is to evaluate the use of a bio-layer interferometry biosensor system from FortéBio as a reliable method for detecting food pathogens in varied matrices.

Methods: Streptavidin-functionalized glass rods with a tip surface area of ~1900 square µm were coated with 25 µg/ml of newly developed biotinylated polyclonal antibodies against either *E. coli* O157:H7, *E. coli* O26, *E. coli* O111 or *Salmonella enterica* serovar Typhimurium. After antibody conjugation, probes were used to sample serial dilutions of bacteria (from 10^2 to 10^9 CFU/ml in 200 µl volumes) suspended in PBS, serum and macerated hamburger extract. Binding events were monitored on a FortéBio Octet Red96 instrument.

Results: Under these conditions, 10^7 CFU/ml were detected in as little as 100-300 seconds. All three antibodies performed in a similar manner and non-specific binding between antibodies and non-target organisms was negligible.

Significance: Bio-layer interferometry is a label-free technique that can be combined with polyclonal antibodies as a biosensor to detect pathogenic bacteria in a manner that is both rapid and specific. With the antibodies that are currently in production at KPL specific for the remaining STEC strains, this multiplexing platform should prove to be a valuable tool in food protection.

P3-16 Rapid Detection of *Escherichia coli* O157:H7 on Turnip Greens Using a Modified Gold Biosensor Combined with Light Microscopic Imaging System

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Introduction: The recent consumer trend toward eating healthy foods has led to an increase in the consumption of fruits and vegetables, resulting in a dramatic increase in *E. coli*-related outbreaks in fresh produce. Therefore, the development of practical and rapid detection methods for *E. coli* O157:H7 is of great interest in produce industry.

Purpose: This research aims to investigate the feasibility of a modified gold biosensor to detect *E. coli* O157:H7 in fresh produce.

Methods: The gold biosensors modified with dithiobis-succinimidyl propionate (Gold-DSP) were immobilized with anti-*E. coli* polyclonal antibodies (pAbs) and *E. coli* O157:H7 bound on Gold-DSP biosensor was vividly detected by a light microscopic imaging system (LMI). The optimum concentration of pAbs, binding efficiency with viable and heat-killed *E. coli* O157:H7, and detection limit of Gold-DSP were determined. Finally, the developed pAbs-immobilized Gold-DSP combined with LMI was applied to turnip greens for rapid detection of *E. coli* O157:H7.

Results: The optimum concentration of pAbs was 200 µg/ml. The detected numbers of viable and heat-killed bacteria on the Gold-DSP were 28.0 ± 2.0 and 2.7 ± 2.0 cells/pixel, respectively. The detection limit of pAbs-immobilized Gold-DSP was 10^4 CFU/sensor. When applied to turnip

greens, the Gold-DSP detected 15.0 ± 5.2 cells/pixel and 154.4 ± 36.7 cells/pixel with the initial populations of 10^1 and 10^2 CFU/ml, respectively, after enrichment for 12 h.

Significance: This research demonstrated that pAbs-immobilized Gold-DSP combined with LMI could be used to rapidly detect *E. coli* O157:H7 on turnip greens in an easy and effective manner.

P3-17 Microbiological Quality Evaluation in UHT Milk, Made by Rapid Method, Reference and ATP Bioluminescence Methods

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Introduction: The standard methods available for microbiological evaluation of UHT milk requires a long time, which prevents prompt corrective action. Therefore, new approaches are needed to quickly indicate possible contamination of UHT milk, among them the technique of ATP bioluminescence.

Purpose: Evaluate ATP bioluminescence 3M™ Microbial Luminescence System (MLS), comparing with traditional media and 3M™ Petrifilm™ Plates for UHT milk products.

Methods: 102 UHT whole milk samples from two manufacturers were incubated for 48, 72, and 168 hours and analyzed for the presence of mesophilic and psychrotrophic microorganisms using Plate Count Agar (PCA), Brain-Heart Infusion (BHI) media and 3M™ Petrifilm™ Aerobic Count (AC) Plates. These data were compared with the results of the 3M™ MLS II.

Results: At the incubation times of 48, 72, and 168 hours; 23.5%, 18.6% and 29.4% of samples were found with aerobic mesophilic counts above the limit established by Brazilian legislation of 100 CFU/mL in PCA, BHI media and 3M™ Petrifilm™ AC plate, respectively. No sample showed psychrotrophic microorganisms counts higher than 100 CFU/mL on PCA and 3M™ Petrifilm™ AC. For the ATP bioluminescence method, 5.9% of the samples at all incubation times, showed values higher than 150 RLU. However, it was noted that at lower reference values (60, 50, 45, and 40 RLU), the percentage of samples identified as positives increased and statistically agreed with aerobic mesophilic microorganisms counts (McNemar test > 0.05). Significant correlations were found between counts of aerobic mesophilic microorganisms on PCA and 3M™ Petrifilm™ AC with the results of ATP bioluminescence technique (Wilcoxon Test - $P \leq 0.05$). There was little variation in the number of positive samples over the incubation periods when using the ATP bioluminescence technique.

Significance: For the dairy industry the 3M™ MLS II System may become an important tool for rapid monitoring of microbiological quality of UHT whole milk to allow product release in a shorter time than that established by the Brazilian legislation.

P3-18 Novel Applications of ATP Bioluminescence for Specific Rapid Microbial Detection

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Introduction: ATP bioluminescence technology is well established and its primary application is the rapid, direct, objective assessment of cleanliness and hygiene through the determination of organic residues. ATP bioluminescence can be used as a non-specific monitor of biomass in a variety of foods, cosmetics, pharmaceutical products and water. A new formulation based on proprietary specific substrates enables this technology for the first time to detect specific bacteria.

Purpose: This paper will describe the novel bioluminogenic tests for coliforms and *E. coli* that will detect low numbers of specific bacteria in 1–7 hours from surface swabs, raw materials and finished products including beverage and water samples.

Methods: The bioluminogenic substrate was challenged with a variety of typical bacteria to determine the specificity and sensitivity. The limit of detection was determined in pure cultures and in real food samples. Naturally contaminated foods were inoculated with typical bacteria to verify detection and to assess the incidence of false positive and false negative detections.

Results: Comparison with tradition cultural methods shows a high degree of agreement in both qualitative and quantitative measurements. The test can also be used for instant colony confirmation. The bioluminogenic assay is robust because there is no interference from background ATP in the sample. Similarly, the early and sensitive detection eliminates the interference for other competing microbes that often cause a problem in traditional cultural microbiological methods.

Significance: The new bioluminogenic assays provide a simple, rapid screening tool for coliforms and *E. coli* and gives results within a working day of 1–7 hours. The technology is capable of detecting and enumerating other bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* through the use of other specific diagnostic substrates. A novel, sensitive, portable, low-cost instrument enables this technology to be used in the laboratory and in remote locations.

P3-19 A Miniaturized Most Probable Number (MPN) Method for the Enumeration of *Campylobacter* spp. from Poultry

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Introduction: Thermophilic campylobacters, such as *C. jejuni* and *C. coli*, account for the majority of cases of human foodborne bacterial gastroenteritis worldwide, with poultry often implicated as the main vehicle of transmission. To accurately assess the efficacy of intervention strategies for the reduction of enteric pathogens in poultry, reliable quantitative methods are required. The most probable number (MPN) technique combines enrichment in multiple tubes across a range of dilutions to establish a numerical data set.

Purpose: To develop and compare a miniaturized most probable number (mMPN) method for the enumeration of *Campylobacter* spp. from poultry associated samples.

Methods: Samples were serially diluted in 96-well dilution plates containing complete Bolton broth supplemented with 25 mg/L sulfamethoxazole (SMX) and, after enrichment, transferred to microtiter plates containing a semi-solidified Bolton broth, SMX and a triphenyltetrazolium chloride (TTC) indicator. The mMPN was compared to standard surface plating on CampyFood ID and Skirrow agars using pure cultures (in triplicate) at low (2.0 log CFU/ml) and high levels (6.0 log CFU/ml) of inocula ($n = 30$), and post-chill commercial broiler carcass rinsates ($n = 34$). Suspect positive (red) wells (mMPN) or typical colonies (standard) were confirmed using BAX® PCR. All media were incubated at 42 °C for up to 48 h under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) and counts converted to log CFU/ml.

Results: For pure cultures, the mMPN method did not differ significantly from direct plating, at the 95% confidence interval (Student's *t*-test $P = 0.38$). For carcasses, there was no significant difference between the mMPN (mean log CFU/ml = 0.76 ± 0.99) and standard plating (mean log CFU/ml = 0.72 ± 0.85), with 97% of samples being less than ± 1 log different (Student's *t*-test $P = 0.47$).

Significance: The mMPN method is a convenient and easily automated process which covers a large range of dilutions, therefore avoiding the need for multiple plates to obtain the most relevant result when using standard plating techniques.

P3-20 Integration of Intestinal Organ Bath Model into Simulator of the Human Intestinal Microbial Ecosystem

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Introduction: Many models have been developed to study gut microbiota. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) is a model that mimics the gastrointestinal tract (GI), which makes it suitable to study gut microbiota. However, the SHIME model is limited by its inability to simulate nutrient absorption as well as the absence of intestinal tissues.

Purpose: This study proposes an integration of an intestinal organ bath system into the SHIME to study the GI tract in vitro, by incorporating animal tissues into different compartments of the system.

Methods: Initially, the SHIME system was set up following Van den Abbeele *et al., Appl Env Microbiol.* 2010; 75(15): 5237-5246, but with six vessels, as per De Boever *et al., Microbial Ecol Health Dis.* 2001; 13: 111-123. Pig intestine and distal colon that were obtained from an abattoir were incorporated at Vessel 3 and 6, representing jejunum/ileum and descending colon, respectively. Instead of a closed fermentor tank system flushed with nitrogen gas, the modified Vessels 3 and 6 became organ bath cultures filled with Kreb-Henseliet buffer, maintained at 37°C and oxygen saturated. Both the small intestine and distal colon were connected through a 6-mm diameter silicone tube with internal PTFE mesh structure. The contents from Vessel 2 (duodenum) and Vessel 5 (transverse colon) were circulated through the lumen and distal colon.

Results: Histological examination of the tissues showed that the mammalian cells retained cells viability for only a short duration. It was also shown that a luminescent strain of *E. coli* attached to the colon but not small intestine during passage through the modified SHIME.

Significance: This novel modification may become an applicable model to study interaction of gut bacteria with the GI tract.

P3-21 Proof-of-concept Chitosan Carbon Dioxide Indicator for Food Packaging

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Introduction: Partial pressure of carbon dioxide (CO_2) in headspace of food packaging is one of major indicators for food quality. CO_2 indicators can be used to monitor food quality which detects the changes of CO_2 level using a visual indicator. Therefore the CO_2 indicator enhances the possibility to provide good quality foods to consumer. The principle of the chitosan-based CO_2 indicator is that insoluble chitosan becomes soluble as the pH of aqueous solution decreases by dissolved CO_2.

Purpose: In this study, pH-dependent solubility and transparency of chitosan aqueous solutions were studied to develop a CO_2 indicator.

Methods: Chitosan aqueous solutions were prepared to investigate their solubility and corresponding transparency at various CO_2 concentrations. Chitosan stock solution was prepared at pH 2 to solubilize it; the transparency of the chitosan solutions at different pH 2, 3, 4, 5, 6, 7, 8 and 9 was measured using spectrophotometry. In addition, the chitosan solutions were stored in an incubator with 100% carbon dioxide gas. Effect of a carbon dioxide absorbent, 2-amino-2-methyl-1-propanol (AMP), on the transparency of chitosan aqueous solutions was investigated at different carbon dioxide levels.

Results: The chitosan solutions were transparent at below pH 7 while cloudy white appearance observed at above pH 7. The pH values of the chitosan solutions prepared at pH 7 with or without 5% AMP were 5.6 and 5.8, respectively, while their transparency values increased up to 99% and 98%, respectively. At pH 8 and 9, the profiles of pH and transparency were similar to those at pH 7 under 100% CO_2 ambient.

Significance: This proof-of-concept CO_2 indicator has a potential to be used for a variety of food packages, such as an indication of food quality in fermented foods and detection of food spoilage.

P3-22 Development of the Roka Automated Molecular Platform™ *Salmonella* spp. Detection System in Food and Environmental Samples Utilizing a Novel Molecular Technology Method

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Introduction: Salmonella has been implicated as a major cause of human foodborne illness worldwide. There is an increased demand to apply effective detection methods that are rapid, accurate and easy to use.

Purpose: To build a *Salmonella* test system for the detection of *Salmonella* spp. in food and environmental samples utilizing a 12–24 hour enrichment time for perishables, and a 24–28 hour enrichment time for non-perishables and environmental samples. The assay utilizes a high throughput, fully automated walk-away instrument system - Roka Automated Molecular Platform™.

Methods: Twenty foods representing 8 different food categories and environmental samples collected from different zones and multiple production plants were enriched for 12–24 hours for perishable foods, and 24–28 hours for non-perishable foods and environmental samples. One broth and one temperature were used for all foods and environmental samples. After enrichment, broth was transferred from the media bag directly to the instrument collection tube. The test method utilized an amplification method targeting Salmonella ribosomal RNA and was compared to a traditional cultural reference method (FDA-BAM). Selectivity was evaluated by testing 100 target microorganisms and 30 non-target microorganisms.

Results: The test method provides a positive result for ≥ 98% of 100 target microorganisms, and a negative result for all 30 non-target microorganisms commonly found in food and grown to a titer ≥ 1 x10^8 CFU/mL. The 20 foods tested demonstrate ≥ 1 CFU detection at 12 -24 hours for perishables and 24–28 hours for non perishable foods and environmental samples. The test method provides the final negative or presumptive positive result in 18–34 hours utilizing a fully automated Instrument system compared to at least 3 days for cultural methods.

Significance: Salmonella spp. can be detected at low levels of contamination in as few as 12 hours of enrichment and combined with the fully automated instrument system offers a rapid, specific, and user friendly test method to monitor and limit contamination issues.

P3-23 Novel Application of Molecular Technologies for Rapid Detection of Food and Environmental Samples by the Roka Automated Molecular Platform™ System

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Introduction: Microorganisms contain 500-10,000 copies of rRNA per cell. Molecular methods that capture and amplify rRNA are highly sensitive and allow rapid detection of microorganisms from food and environmental samples.

Purpose: To describe the novel application of three technologies, specific target capture, transcription mediated amplification and acridinium ester-based chemiluminescence to allow rapid detection of pathogens from food and environmental samples. A fully automated instrument platform that captures, amplifies and detects rRNA from pathogens was used.

Methods: A variety of food and environmental samples were inoculated with a single cell of Salmonella or Listeria and enriched for 24 hours or less. An aliquot was placed in a sample collection tube containing lysis buffer. The sample was then placed into the Atlas system which carried out target capture, amplification and detection of the rRNA from either pathogen.

Results: For both pathogens, a single inoculated cell could be detected. Use of rRNA allowed for sub cfu detection of pathogens in the presence of high background flora; specific target capture eliminated inhibitors found in food products and transcription mediated amplification allowed rapid and efficient amplification of target.

Significance: The use of proprietary molecular technologies allows the detection of single cells of Salmonella and Listeria from environmental and food samples in 24 hours or less on a high-throughput fully automated platform.

P3-24 Development of a Detection Method for *Listeria* spp. in Food and Environmental Samples on Roka Automated Molecular Platform™, a Fully Automated Walk-away System

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Introduction: Listeria is an environmentally ubiquitous organism implicated as a major cause of human foodborne illness worldwide. *Listeria*'s long lag phase and hindered growth in the presence of competitive flora present a challenge for rapid and reliable detection in food and environmental samples with a single 24-hour enrichment step.

Purpose: The purpose of the study is to develop a sensitive and reliable detection method for *Listeria* with testing being performed after a single 24-hour enrichment for both food and environmental samples. The assay utilizes a high throughput, fully automated, walk-away instrument, the Roka Automated Molecular Platform™.

Methods: Environmental samples collected from several food pilot plants and 9 different foods were analyzed and compared to a culture method (USDA, FDA-BAM or similar). Half-Fraser media was added to each food and environmental sample, incubated at 35°C for ≤ 24 hours, a sample transferred from the media bag to a collection tube containing a lysis reagent, and then loaded onto the instrument. Selectivity was evaluated by testing 50 target microorganisms and 30 non-target microorganisms.

Results: The test method provided a positive result for ≥ 98% of 50 target microorganisms, and a negative result for 30 non-target microorganisms commonly found in food and grown to a titer ≥ 1E+08 CFU/mL. The results demonstrated ≥ 1 CFU detection at ≤ 24–28 hours for 9 foods and environmental samples. The test method provided the final negative or presumptive positive result in ≤ 24–28 hours utilizing the fully automated system compared to at least 3 days for culture methods.

Significance: Listeria spp. can be detected at low levels of contamination at ≤ 24–28 hours of enrichment and the fully automated instrument offers a rapid, specific, and user friendly test method to monitor and limit contamination issues.

P3-25 Optimization for the Removal of *Salmonella, Escherichia coli* O157:H7 and *E. coli* O157:H12 from Water Using Zero-valent Iron

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Introduction: Microorganisms such as *E. coli* O157 and *Salmonella* may be detected in surface and ground water used for irrigation and thus pose a food safety risk. Foodborne outbreaks have been documented to be caused by pathogenic *E. coli* in irrigation water. Recent studies have shown that zero-valent iron (ZVI) nanoparticles can be used to remove and inactivate *E. coli*.

Purpose: To evaluate the removal and inactivation of *E. coli* O157 and *Salmonella* Newport by filtration using a combination of sand and ZVI.

Methods: Salmonella Newport, *E. coli* O157:H7 (strain 4407) and *E. coli* O157:H12 were separately prepared and inoculated into artificial groundwater (AGW). A sand-only control column was packed with 10 cm of sand only and experimental columns were packed with layers of 50:50 ZVI-sand of 4 cm, 2 cm x 2, or 7 cm. Inoculated AGW was applied to all columns at a constant flow rate of ~1ml/min and outflow was collected as 120 x 5 ml samples and enumerated on TSA + nalidixic acid or XLT-4 agar. Data were analyzed by calculating total removal for each column.

Results: For each sand or iron column, 300 ml of ~1×10^8 CFU/ml *E. coli* and *Salmonella* was added, and no removal was observed for control sand columns for either *E. coli* O157 or *Salmonella*. Approximately 2 log CFU *E. coli* O157:H12 was removed by ZVI filtration; however, ZVI had less effect on *E. coli* O157:H7 with ~ 0.6 log of initial removal and increased to >5 log after 90 days with 7cm iron in one trial. Removal of *Salmonella* was initially 0.6 log. The zeta potential of *E. coli* O157:H12 was -27.7(±1), and -12.2(±4.8), -12.2(±4.9) for *E. coli* O157:H7 and *Salmonella* Newport, respectively. The *E. coli* O157:H12 was significantly more negatively charged possibly leading to higher removal by ZVI compared to the other two bacteria.

Significance: These results indicate that ZVI can remove *E. coli* O157 and *Salmonella* from water, and may be more effective against bacterial cells with greater negative surface charges.

P3-26 Thermal Resistance of *Salmonella* in Desiccation and Rehydration

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Introduction: Survival of salmonellae in dehydrated foods and dry processing environments is a concern. The desiccated bacteria become rehydrated when they contaminate foods formulated to higher water activities. How rehydration in a new matrix affects physiology and thermal behavior remains understudied.

Purpose: Characterize the impact of desiccation and rehydration on thermal inactivation of *Salmonella*.

Methods: S. enterica Tennessee was grown in TSB at 37°C for 20 h. Desiccation and rehydration were achieved by storing bacteria at a_w 0.11, followed by transfer to higher equilibrated relative humidity (ERH) of 33, 55, 84, or 97%. The cells were treated at 50, 60, 70, 80, and 90°C for 10 min, and then enumerated on TSA. The impact of desiccation duration on thermal resistance was also measured by 10-min 60°C treatment of cells stored at a_w 0.11 for 1, 2 or 3 weeks.

Results: Thermal resistance of desiccated cells was inversely correlated to a_w: for example, desiccation at a_w 0.11 to 0.97 resulted in 0.5 to 3.3 log reduction, respectively, after 60°C treatment. Once cells were equilibrated at 11% ERH, continual storage for three weeks did not further increase the thermal inactivation efficiency. When desiccated cells were partially re-hydrated, thermal inactivation efficiency was restored in proportion to the a_w of the reconstituted cells. In contrast, full rehydration with PBS led to complete inactivation after 60°C heat treatment (> 7 log reduction).

Significance: These results indicate that a water-based heat treatment may be more effective than dry heat for inactivating desiccated *Salmonella* in sanitization processes.

P3-27 Single Cell Variability and Population Dynamics of *Listeria monocytogenes* and *Salmonella* Typhimurium in Fresh-cut Salads and Their Sterile Liquid or Solidified Extracts

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Introduction: Leafy vegetables are associated with many foodborne outbreaks worldwide, due to contamination with pathogens pre- or post-harvesting. Especially during the preparation of ready-to-eat (RTE) salads, nutrients released from the cut tissues may enhance growth of microbial cells and potentially compromise the safety of these foods.

Purpose: The study aimed to evaluate the growth variability of single cells of *Listeria monocytogenes* and *Salmonella* Typhimurium, as compared to higher populations, in leafy vegetable salads as well as in their sterile extracts.

Methods: Samples (10 g) of freshly cut lettuce or cabbage, previously treated with 200 ppm of chlorine for 10 minutes, were inoculated with 1–3 or 1000 cells of *Listeria monocytogenes* Scott A or *Salmonella* Typhimurium and stored at 8°C. In addition, liquid or solidified sterile extracts of lettuce and cabbage were also inoculated with the same levels of the above pathogens, in order to study the behavior of these organisms in the presence or absence of indigenous flora. The sterile media were stored at 8 or 10°C, simulating temperature fluctuations above a common refrigeration temperature in summer.

Results: Growth of the high inoculation level occurred up to 3 logs with limited variation (SD <1 log CFU/g). Conversely, great variability (from <0.5 to 3 log CFU/g increase) in the growth of single cells of *L. monocytogenes* or *S.* Typhimurium was observed on lettuce and cabbage; however, such variability was not observed in their sterile extracts. Lettuce was a more favorable environment for growth of both pathogens, compared to cabbage, which did not support growth of *Salmonella*. Notably, single cells of *L. monocytogenes* could not initiate growth in sterile cabbage extracts; whereas, they showed variable increases from 1 to 3.5 logs in cabbage bearing the natural flora. Increasing storage temperature by 2°C markedly enhanced growth of pathogens, especially of *Salmonella*. The latter showed no growth at 8°C but increased 4 logs at 10°C.

Significance: Growth of pathogens in leafy greens salads depends on the indigenous microflora, the storage temperature and the available nutrients. Such results may be useful in the exposure assessment of pathogens in RTE leafy salads and contribute to the improvement of safety interventions.

P3-28 Inactivation of *Salmonella* in Bakery Products under Non-isothermal Conditions

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Introduction: Evidence that *Salmonella* has increased thermal stability under low-moisture conditions and recent outbreaks associated with nuts and seeds have raised concerns about the efficacy of baking as a means to inactivate *Salmonella*.

Purpose: The objective of this study was to determine the inactivation of *Salmonella* during baking of inoculated croutons, pecans, and sesame seeds on baked goods.

Methods: Sesame seeds, pecans and bread cubes were inoculated with 6 to 8 log CFU/g *Salmonella enterica* (5-serotypes) and air dried for up to 6 hours to achieve target water activity (a_w). Bread cubes were baked at 127°C for 15 minutes, sesame seeds were added to the surface of leavened dinner roll dough and baked at 163°C for 17 minutes and pecans on the surface of a filled pie crust were baked at 177°C for 45 minutes. Duplicate samples were removed at designated intervals (0-time, minimum two intermediate times, end of bake and over bake) and plated on XLD with a thin TSA overlay. Each treatment was replicated at least twice.

Results: a_w decreased from 0.95, 0.71 and 0.83 at 0-time to 0.36, 0.21, and 0.57 at end of bake in croutons, sesame seeds, and pecans, respectively. End of bake temperature was 110, 146, and 143°C, respectively, with 3.1, 3.8, and >5 log reduction for croutons, sesame seeds and pecans. Baking time and temperature correlated with a_w reduction but exposure time to surface temperatures exceeding 100°C had the most significant effect on log reduction.

Significance: This study confirmed that inactivation rates of *Salmonella* in reduced a_w bakery products were observable longer than for high moisture foods, but typical baking procedures generate at least a 3-log reduction of the pathogen. Use of ingredients with good microbial quality and environmental controls are needed to ensure the safety of low a_w bakery products.

P3-29 Antimicrobial Susceptibility, Virulence Genes and PFGE Patterns in *Salmonella* spp. Isolated in a Brazilian Slaughterhouse That Produces Meat for Export

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Introduction: Latent zoonoses are caused by microorganisms that do not cause any pathology in the harboring animal (reservoirs). Once present in one animal, these microorganisms can contaminate the whole meat production chain. The most relevant pathogen in bovine meat is *Salmonella* spp., the major causative agent of foodborne diseases in the world. Several studies have shown that *Salmonella* spp. can occur in raw bovine meat at retail level, but little is known about this pathogen in meat produced for export.

Purpose: The aims of the present study were to evaluate the prevalence of *Salmonella* spp. in a large Brazilian slaughterhouse that produces meat for export, check for antimicrobial susceptibility, virulence genes and PFGE patterns of the strains isolated at different points of the production chain.

Methods: The survey was conducted in a large slaughterhouse that produces bovine meat for export, located in the State of São Paulo, Brazil. Surface samples were collected from 200 animals, at three points of the slaughtering process: hide right after bleeding (CO), carcass after removal of the hide (CA1) and carcass after cleaning but before chilling (CA2). The ISO 6579:2002 isolation method and confirmation by PCR were used for detection and identification of *Salmonella* spp. Serotyping was carried out in the Enterobacteriaceae Reference Laboratory of Instituto Oswaldo Cruz, RJ, Brazil. The strains were tested for antimicrobial susceptibility according to the CLSI, and for the presence of virulence genes *invA*, *sitC*, *spaN*, *sifA* and *msgA* by PCR. Molecular typing by PFGE was performed according to the protocol recommended by the *Salmonella* PulseNet.

Results: Salmonella spp were found in the hide (CO) of 31 animals (15.5%). Seven (3.5%) and six (3.0%) carcasses were positive at points CA1 and CA2, respectively. Two animals were positive in the hide and point CA2, simultaneously, and only one was positive at the three points, simultaneously. There was a prevalence of serotype *S.* Infantis (54.5%), followed by *S.* Enteritidis (13.6%). All strains were sensitive to cefotaxime, ciprofloxacin, gentamicin and imipenem, whereas five (11.4%) were resistant to ampicillin and tetracycline simultaneously. The five investigated virulence genes were detected in 52.2% of the isolates. PFGE indicated that the 44 tested strains belonged to 27 clusters, and 18 (66.7%) were constituted of one single strain.

Significance: To our knowledge, this is the first report on *Salmonella* spp in a Brazilian slaughterhouse that produces meat for export.

Acknowledgment: FAPESP.

P3-30 Influence of Carvacrol and Cinnamaldehyde on the Thermal Inactivation of Multiple *Salmonella* Serotypes in Ground Chicken

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Introduction: The use of heat to inactivate foodborne pathogens is a critical control point and the most common means of assuring the microbiological safety of processed foods. A key to optimization of the heating step is defining the heat resistance of target pathogens.

Purpose: We investigated the heat resistance of an eight strain cocktail of *Salmonella* spp. in chicken supplemented with carvacrol (CR; 0 - 1.0%, w/w) and cinnamaldehyde (CN; 0 - 1.0%, w/w).

Methods: Inoculated meat was packaged in bags which were completely immersed in a circulating water bath and held at 60, 65, and 71.1°C for predetermined lengths of time. The recovery medium was tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. D-values (time, in minutes, required for the bacterial population to decrease by 90%) were calculated by fitting a survival model to the data with a curve fitting program.

Results: In the control chicken, D-values ranged from 2.34 min at 60°C to 0.01 min at 71.1°C. A significant increase ($P < 0.05$) in the sensitivity of the bacteria to heat was observed with the addition of 0.1% CR or 0.5% CN at 60°C. While 0.1% CR in chicken at 65°C significantly decreased ($P < 0.05$) the D-values from 0.74 to 0.22 min, the values were not significantly different ($P > 0.05$) with added CN. The effect of the added antimicrobials in rendering the pathogen more sensitive to the lethal effect of heat was not observed at 71.1°C.

Significance: Thermal death times from this study will assist the retail food industry to design cooking regimes that ensure the safety of chicken contaminated with *Salmonella*.

P3-31 Rdar Morphotype and Its Relationship to Desiccation Tolerance in *Salmonella* spp.

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Introduction: Salmonella strains that produce thin aggregative fimbriae and cellulose when grown on LB no salt (LBNS) agar produce red, dry, and rough (rdar) colonies. Previous studies have shown that the rdar-positive morphotype (rdar+) is linked to environmental stress resistance including tolerance to desiccation.

Purpose: The purpose of this study was to evaluate the relationship between rdar morphotype and desiccation tolerance in *Salmonella* spp.

Methods: Salmonella Enteritidis PT30 (SEPT30) and *Salmonella* Senftenberg 775W (SS775W), representing rdar+ and rdar-negative (rdar-) morphotypes, respectively, were evaluated in a model system. Isolates were grown in either broth or on agar plates, suspended in Butterfield's phosphate buffer, and stored at 70% relative humidity (RH) on glass coverslips. Surviving cells were enumerated on tryptic soy (TSA) and XLD agars. The influence of nutrient availability and osmolarity on production of cellulose and thin fimbriae were evaluated using 0.1% LB and LBNS. Cellulose and thin fimbriae were measured by standard methods and the up-regulation of the cellulose regulator gene (*adr*A) was examined by real-time reverse transcriptase PCR.

Results: Desiccation survival (reduction over 48 h) and short-term persistence (7 days) of SEPT30 grown on agar plates was significantly better that SEPT30 cultured in broth or SS775W cultured in either broth or on plates. SEPT30 but not SS775W produced significantly greater amounts of cellulose when grown on agar plates compared to broth. In contrast, significantly greater amounts of thin fimbriae were produced in broth culture. Compared to growth in broth, *adr*A was upregulated in cells grown on agar (8-fold), under limited nutrients (0.1% LB; 10-fold) and low osmolarity (LB without salt; 20-fold).

Significance: These findings will be useful in developing strain and culture preparation methods for conducting food safety challenge studies and provide insight into survival and persistence of *Salmonella* in low-moisture foods.

P3-32 Comparison of the Dry Heat Sensitivity of *Salmonella enterica* on Alfalfa Seeds Stored at Different Temperatures

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Introduction: Sprouts eaten raw are increasingly being perceived as hazardous foods. They have been implicated in several outbreaks of salmonellosis where the seeds were found to be the likely source of contamination.

Purpose: The objectives of this study were to (i) determine the influence of storage temperature on the dry heat sensitivity of *Salmonella enterica* on alfalfa seeds and (ii) identify effective heat treatment conditions that can eliminate the pathogen at minimal expense to the seed viability.

Methods: Alfalfa seeds were inoculated with a 6-strain composite of *Salmonella* to a level of ~ 6 log CFU/g and stored for 7 days at -18, -10, 0, 4, 10, 15 or 22°C. Seeds were then dry heated at 60°C for 24-96 h, 65°C for 2-8 h and 70°C for 1-4 hours. Seeds were then chilled and microbiologically analyzed by plating on TSAYE.

Results: A decline of < 1 log CFU/g was observed for seeds only stored at the different temperatures. The heat sensitivity of *Salmonella* was dependent on the storage temperature and increased in the order of 0°C > -18°C > 4°C > 10°C ≈ -10°C > 15°C > 22°C. Storage at -18°C or 0°C followed by dry heat at 60, 65 and 70°C for 60, 3 and 1 h respectively, consistently eliminated the pathogen (< 1 CFU/2 g). However, a variable surviving population (2.8 – 4.6 log CFU/g) was detected for seeds stored at the other storage temperatures and subjected to the same heat treatments. Storage of uninoculated seeds at -18°C or 0°C followed by heating at 60°C for 60 h did not significantly ($P > 0.05$) reduce the germination rates (95-96%) and sprouting yield (12.4 – 12.5 g/g) of seeds compared to untreated controls.

Significance: These observations will be useful when developing effective strategies and practices to enhance the microbiological safety of alfalfa sprouts.

P3-33 Source of *Salmonella* in Ground Beef from Non-fed Beef

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Introduction: Salmonella spp. are major foodborne pathogens, and were the reason for more ground beef recalls in 2009 than *E. coli* O157. Additionally, antibiotic resistance is increasing. Identifying the sources of *Salmonella* from cattle should enable beef processors to limit its prevalence in ground beef.

Purpose: To determine the source(s) of *Salmonella* found in ground beef and the antibiotic-resistance profile.

Methods: One hundred U.S. dairy cows were harvested and samples were collected from hides, carcasses after hide removal before intervention and after intervention, superficial cervical lymph nodes from the chuck, and trim. The trim from these carcasses was ground separately and sampled. Air samples were also collected from processing plant areas. The presence of *Salmonella* was determined using PCR. For source attribution, all *Salmonella*-positive samples were subjected to Pulsed-Field Gel Electrophoresis. Antibiotic resistance was assessed by the Vitek Gram Negative Susceptibility Card.

Results: Salmonella was detected by PCR in 164/457 samples (35.67%). Eight DNA *Xba* I restriction patterns were observed by PFGE analysis. The majority of the isolates had restriction digest patterns (RDPs) B and E. The strain isolated from ground beef was indistinguishable from hide and carcasses before intervention. The *Salmonella* isolated from trim samples and the lymph nodes matched in the RDP F. The ground beef isolate was resistant to Amoxicillin/CA, Ampicillin, Cefazolin, Ceftazidime and Trimethoprim-Sulfa, while the trim isolate was not resistant to any antibiotic. No *Salmonella* were detected from any of the air samples.

Significance: These results indicate that trim and lymph nodes are the most likely sources of *Salmonella* in ground beef. Effective dressing practices to reduce transfer of bacteria from hide to carcass and elimination of lymph nodes from raw ground beef components should control *Salmonella* contamination. Because total elimination of lymph nodes is not practical or possible, other avenues should be explored.

P3-34 Survival of *Salmonella* Strains in Acetic Acid and Rice Vinegar in Relation to the Biofilm-formation Capability

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Introduction: Organic acids have been used for preservation of foods and washing vegetables and utensils. Like many other bacteria, survival of *Salmonella* can be controlled by acid treatment. We have previously found *Salmonella* strains having different abilities to form biofilms, and that the biofilm-formation capability may enhance the survival under various stresses like acid.

Purpose: The purpose of this study was to gain insights into the relationship of the biofilm-formation capability and survival of *Salmonella* strains under acidic conditions.

Methods: Eight strains of *Salmonella* having high and low biofilm-formation capability were used. One ml of incubation culture which reached the stationary phase (reached 10^8 CFU/ml) was suspended in 9 ml of phosphate buffered saline (adjusted to pH 3.0 with HCl or pH 3.9 with acetic acid) and rice vinegar on the market diluted 1:15 with distilled water (adjusted to pH 3.9). Surviving cells were enumerated using tryptic soy agar. Survival curves were plotted and fitted to the Weibull model using GInaFiT software.

Results: The results from the Weibull model indicated obvious differences in survival between the two groups of strains having different abilities to form biofilm in acetic acid and rice vinegar, but not in HCl. The number ($\log_{10}$ CFU) of surviving cells after 48 h exposure to acetic acid, rice vinegar and HCl were 2.83 ± 0.34 vs. 1.91 ± 0.42, 4.12 ± 0.20 vs. 2.03 ± 0.52 and 3.73 ± 0.57 vs. 2.9 ± 1.89, respectively, for high vs. low biofilm-formation capability. The delta values (first decimal reduction time) of the model were 9.96 vs. 3.04, 11.33 vs. 4.35 and 7.71 vs. 6.40, respectively.

Significance: These data suggest that the biofilm-formation capability of *Salmonella* may aid the survival of the cells in acidic media, especially in acetic acid and rice vinegar.

P3-35 Differences in the Thermal Resistance of *Salmonella* Tennessee and Oranienburg in Peanut Butter Related to Growth as Sessile or Planctonic Cells

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Introduction: The thermal destruction of *Salmonella* species in peanut butter is non-linear deviating from expected pseudo-first order thermal destruction kinetics. Reasons for deviations from pseudo-first order kinetics are unknown, but may be a result of the physiological state of the organism which is influenced by state/type of growth.

Purpose: Inoculation of *Salmonella* species into a low-water activity product may induce immediate physiological changes in the microorganism particularly after growth in a high-water activity environment. Growth on a solid matrix may result in a different physiological state more similar to low-water activity environments thus avoiding such changes. This study examines differences in survival in peanut butter based on sessile vs. planctonic cell growth.

Methods: Salmonella Tennessee and Oranienburg were grown 24 hrs aerobically in trypticase soy broth and on trypticase soy agar at 37°C then harvested and inoculated into peanut butter. Peanut butter was held at 25°C and tested for *Salmonella* concentration immediately after inoculation and at various intervals up to two weeks. Thermal resistance was measured at 85°C immediately after inoculation.

Results: Both strains showed similar thermal resistance in peanut butter, regardless of growth as sessile or planctonic cells. However, thermal destruction curves from sessile cultures show less curvature (are more linear) than those obtained using planctonic cells ($P = 0.0198$ and $P = 0.0047$ for Oranienburg and Tennessee, respectively). In addition, both *Salmonella* strains showed significantly more stability in peanut butter when originally grown on solid media ($P = 0.001$), with a less then 1-log loss over two weeks as opposed to a 1- to 2-log loss when grown in liquid culture.

Significance: Use of *Salmonella* species grown on solid media may result in reduced physiological changes that occur when moving cells from high- to low-water activity environments. As a consequence, results for thermal inactivation may more accurately assess survival in low-water activity environments.

P3-36 Comparative Expression Array Analysis of *Salmonella* Desiccation Resistance Mechanism

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Introduction: The survival of *Salmonella* in dry foods has caused multi-state outbreaks. The underlying mechanism of *Salmonella* desiccation survival remains unclear.

Purpose: Understand the molecular mechanism of *Salmonella* desiccation survival.

Methods: Salmonella Typhimurium LT2 and Tennessee, each representing a weak and strong desiccation-resistant strain, respectively, were grown in TSB from a single colony for 20 hours. Desiccation treatment was achieved by incubating cells on filter discs in a desiccator (11% ERH) at 25°C for 2 hours. Total RNAs in both desiccated/non-desiccated conditions were isolated and normalized in three independent experiments. Total 17K probes including two probes for each ORF from both genomes were printed on each slide. Hybridization was processed at Genus System Inc, IL (Northbrook, IL). Signals of all probes were normalized to the 75th percentile of each array. Data validation was done with real time PCR. Gene Spring software (Agilent Inc., CA) was used for data analysis.

Results: Differential expressions of genes in both genomes were profoundly triggered by desiccation, but at greater extent and with more genes up-regulated in Tennessee than LT2. With a cut off at 5 folds, about 106 and 86 genes were up-regulated in Tennessee and LT2 each; about 20 and 21 genes were down regulated in Tennessee and LT2, respectively. The gene expression fold changes in each genome ranged from +93 to -13 in Tennessee and +64 to -14 folds in LT2 respectively. Both genomes shared similar primary pathways that were possibly involved in desiccation survival. Fatty acid degradation, carbon metabolism, osmotic pressure protection, oxidation response and cell membrane integrity were all up-regulated during exposure to desiccation. Nucleotide synthesis, DNA polymerase, DNA replication initiation, ribosome synthesis, and tRNA synthesis were all down-regulated.

Significance: The knowledge gained from mapping the global gene desiccation response will direct future studies and lead to more effective intervention of *Salmonella*.

P3-37 Characterization and Antibiotic Resistance Profiles of *Salmonella* spp. Isolated in Pork and Poultry Plants

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Introduction: Antimicrobial resistance associated with *Salmonella* is an important public health concern. Cross-contamination can occur throughout vertically integrated meat processing operations and presents a challenge in finding control measures. Serotyping and genetic profiling of both resistant and non-resistant strains isolated along the production chain is essential in identifying the risk factors for cross-contamination.

Purpose: The objective of this project was to investigate the prevalence of individual serotypes, genotypes, and antibiotic resistance profiles of *Salmonella* isolates recovered from commercial pork and poultry sources and to identify areas of potential risk in contamination. Using a combination of traditional and rapid methods, unique profiles of *Salmonella* isolates were obtained and compared.

Methods: Fifty *Salmonella* isolates each from pork and poultry were collected using a modified version of the USDA method and serotyped according to the traditional Kaufmann-White scheme. For comparison, these isolates were then rapidly serotyped using the PTS system which yields unique microarray hybridization profiles. Cultures were then genotyped by PFGE according to the CDC protocol for *Salmonella* using Xba1 restriction enzyme. Antibiotic resistance to six different antibiotics was measured via the Kirby-Bauer method and Flash and Go Advanced Inhibition Zone Measuring software.

Results: Seven isolates from pork sources were resistant to tetracycline while two isolates were found to have multidrug resistance. No resistance was found in isolates from poultry. Seventeen different serotypes were represented in the isolates and macrorestriction with XbaI distinguished 18 genotypes. Ten clusters were generated, eight from pork and two from poultry. Isolates from the same host and collected from the same plant were clustered together indicating the high specificity of the serotypes to a specific host and environment. *S.* Braenderup and *S.* Kentucky represented the most frequently isolated serotypes from poultry, while *S.* Ohio and *S.* Anatum were most frequently isolated from pork.

Significance: Traditional methods used in combination with newer rapid methods work well in providing supporting information for verifying the accuracy of the data collected. In this study, the PFGE profiles helped in the discrimination of mismatches encountered between traditional serotyping with the Kauffman-White method and the PTS system, while at the same time generating a profile of strains found in the processing plants. Antibiotic susceptibility testing showed that multi-drug resistance continues to be a concern, particularly in pork processing environments.

P3-38 Transfer and Recovery of *Salmonella* Species from Contaminated Nut Butters to Food Contact Surfaces

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Introduction: Salmonella species can survive and/or persist for long periods of time in low-moisture foods and on food contact surfaces. Understanding parameters of transfer and survival can provide information required for successful control of this foodborne microorganism.

Purpose: This study investigated the recovery of *Salmonella* from food contact surfaces such as stainless steel, delrin, polyethylene and polyurethane as well as their transfer from nut butters onto the contact surfaces.

Methods: Salmonella Tennessee and Oranienburg were grown overnight to stationary phase, harvested and inoculated separately into either peanut butter or almond butter. Four different food contact materials were obtained as squares sized 10 x10 cm each. One gram of inoculated nut butter was applied to each of the three separate 4 x 4 cm areas on the materials and stored at room temperature. The nut butters were removed after 1, 7 and 14 day intervals to leave a visually clean surface. Surfaces were swabbed and/or tested with contact plates to determine the population of any remaining cells.

Results: The transfer rates of the strains onto all the materials were approximately 2.85×10^7/ 1.90×10^8 CFU/ml for Tennessee and 2.45×10^7/7.30×10^7 CFU/ml for Oranienburg. Populations of *S.* Tennessee applied through peanut butter and almond butter decreased >1 log on food contact surfaces after each week; whereas, *S.* Oranienburg decreased by 3 logs the first day, after which it remained constant in 2 weeks. Trials with almond butter showed 1 log reductions at each time period tested. The survival of both the strains was highest on polyurethane.

Significance: These studies contribute to a better understanding of the behavior of *Salmonella* Tennessee and Oranienburg in nut butter and on food contact surfaces. Estimates of contamination levels may be determined from transfer rates for *Salmonella* from contaminated to uncontaminated foods and food contact surfaces.

P3-39 Strain, Type of Food-conditioning Film and Their Interaction Significantly Affect Cell Density and Biofilm Formation by *Listeria monocytogenes*

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Introduction: Specific strains of *Listeria monocytogenes* are known to persist in different food processing plants for years and cause contamination, but there is a lack of knowledge of the mechanisms of their persistence.

Purpose: The purpose of the study was to investigate the effects of type of strain of *L. monocytogenes* and type of food conditioning film (CF) on cell density and biofilm formation on glass slides.

Methods: Seven strains of *L. monocytogenes* were used in the study, including two strains lacking the comK prophage (a Lineage III strain & an Epidemic Clone I (ECI) strain) and five strains containing the comK prophage (three ECII strains, an ECIII strain & an ECV strain). Five RTE foods were used to make CFs, including soft cheese, hot dog, ham, cooked turkey and chicken. Briefly, each food was blended with water and the resulting food slurry was dried to form CF on slides with removable chambers. Broth cultures of different strains were loaded on these CFs. After incubation and staining, slides were examined using fluorescence microscopy.

Results: Type of strain and CF and the interaction between the two significantly affected cell density on slides ($P < 0.001$). Strains lacking the comK prophage showed lower cell densities than those containing the prophage on all four meat and poultry CFs ($P < 0.05$). The ECV strain

produced the highest cell densities and mature biofilms on all CFs except cheese. Among all the CFs, chicken produced the highest average cell density across all the strains ($P < 0.05$), followed by ham, turkey, hot dog and cheese. Food conditioning films were visible when cell densities were low or moderate; no food conditioning films were left when heavy biofilms were formed by some strains.

Significance: The results indicate that the comK prophage may contain genes essential for attachment and growth of *L. monocytogenes* on meat and poultry CFs. Biofilms formed by the strains containing this prophage may protect cells and help specific strains persist in meat and poultry processing plants for long periods.

P3-40 Occurrence and Characterization of *Listeria* spp. in Ready-to-Eat Retail Foods from Vancouver, British Columbia

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Introduction: Listeria monocytogenes (Lm) is a ubiquitous microorganism capable of causing severe disease in immunocompromised individuals consuming contaminated ready-to-eat (RTE) products. Control of Lm has been challenging for food producers, with failures leading to contaminated foods. Recent data from British Columbia (BC) suggest issues with Lm control in RTE food production sector, though data demonstrating contamination in retail products are lacking.

Purpose: The purpose of this project was two-fold; (i) determine the occurrence of *Listeria* spp. and Lm in retail RTE meat and fish products in Vancouver, BC; (ii) for recovered Lm, subject the isolates to phenotypic and genotypic analyses to characterize their virulence potential.

Methods: Deli meat (n=40) and fish (n=40) samples from 17 stores were tested for *Listeria* spp. using conventional methods. Three isolates from Lm-positive samples were serotyped, subjected to PFGE, and screened by PCR for the presence of LGI1 genomic island. Antimicrobial resistance of Lm isolates was determined using disc diffusion assay according to CLSI guidelines.

Results: No RTE meat samples tested positive for *Listeria* spp., while 8 (20%) smoked fish samples were contaminated. Lm was recovered from 2 (5%) smoked salmon products, while *L. innocua* and *L. welshimeri* were found in 4 (10%) and 2 (5%) samples, respectively. Lm isolates belonged to 1/2a and 1/2b serotypes and possessed dissimilar PFGE types. LGI1 was detected in three 1/2a Lm clonal isolates recovered from smoked salmon. All Lm isolates possessed resistance to cefoxitin, clindamycin, nalidixic acid and had reduced susceptibility to ciprofloxacin. Two Lm 1/2b clonal isolates were resistant to streptomycin with one being additionally resistant to amikacin and the other exhibiting reduced susceptibility to amikacin.

Significance: Listeria spp., including Lm 1/2a serotype, can be found in RTE fish products in BC. Antimicrobial resistance, coupled with the potential for human disease linked to 1/2a serotypes and the presence of LGI1 in Lm isolates is concerning. These findings highlight the need for better control and monitoring of Lm in RTE products.

P3-41 Occurrence and Distribution of *Listeria* spp. in Facilities Producing Ready-to-Eat Foods under Provincial Inspection Authority in British Columbia, Canada

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Introduction: Food-processing facilities contaminated with *Listeria monocytogenes* (Lm), the major causative agent of human listeriosis, pose a potential food safety risk. The recent Canada-wide listeriosis outbreak associated with ready-to-eat (RTE) meats has reinforced the importance of monitoring and controlling Lm through the chain of food production in the country. In the province of British Columbia (B.C.), sampling for Lm in food and processing environments is not required for non-federally registered producers.

Purpose: As limited data exist on the occurrence of *Listeria* spp. in food and food production environments in B.C., a survey was conducted to estimate the prevalence of *Listeria* spp. and Lm in RTE foods and environmental swabs from dairy, fish and meat facilities subject to provincial inspection.

Methods: In total, 262 RTE food and 305 environmental swab samples were collected from 53 food-processing facilities from August to October 2009. Environmental swabs and food samples were analyzed using Health Canada's standard culture methods.

Results: Overall contamination with *Listeria* spp. and Lm in foods was 9% (23/262) and 5% (14/262), respectively. Fish products from five of 12 fish processors surveyed were contaminated with Lm, while the pathogen was not found in RTE products collected from 17 dairy and 14 meat processors. *Listeria* spp. and Lm, respectively, were found on 30% and 13% of non-food contact surfaces, 5% and 4% of close-to-food surfaces, and 6% and 3% of food contact surfaces. Analysis by facility category showed the highest rate of environmental contamination with Lm in fish (38%) facilities when compared with dairy (18%) and meat (14%) processors.

Significance: The results suggest that current practices for the control of Lm in B.C. inspected dairy and meat facilities are effective in limiting food contamination with Lm. However, there is a lack of control for Lm in RTE fish processing facilities under provincial inspection authority.

P3-42 Ecology of *Listeria monocytogenes* in the Retail Deli Environment

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Introduction: Efforts to improve food safety using a farm-to-fork approach require comprehensive data on pathogen ecology throughout the food chain. While *Listeria monocytogenes* is commonly found in many natural and man-made environments, understanding of the ecology of *L. monocytogenes* in retail deli environments remains limited. This study was initiated at the request of USDA/FSIS and as findings of comparative *L. monocytogenes* risk assessments identified a need for better understanding of *L. monocytogenes* presence and persistence in the retail grocery store environment.

Purpose: A longitudinal study using conventional detection methods and molecular subtyping methods was conducted to enhance stakeholders' understanding of the ecology of *L. monocytogenes* on food and non-food contact surfaces in retail establishments.

Methods: A longitudinal study in 30 retail stores in 3 US states was conducted. In Phase I of this study, 5 stores in each state were sampled prior to daily operation once a month for 3 months for *L. monocytogenes*. In Phase II, 24 to 28 food contact and non-food contact sites were sampled in each of 30 stores during daily operation for 6 months.

Results: L. monocytogenes were isolated from 8 of the 15 stores sampled during Phase I. Among 3574 sites sampled during Phase II, 9.6% were positive for *L. monocytogenes*; 16 of 30 stores showed *L. monocytogenes* prevalence <1% for all food contact surfaces. Pulsed-Field Gel Electrophoresis (PFGE) subtyping revealed considerable subtypes diversity among isolates. Re-isolation of identical PFGE types over time in different non-food contact surfaces suggested persistence or re-introduction in some stores. Interestingly, PFGE patterns for isolates from non-food contact surfaces in some stores were distinct from PFGE patterns for occasional food contact surface isolates suggesting limited cross-contamination between these sites.

Significance: This study provides data on *L. monocytogenes* prevalence and persistence in retail delis and will facilitate development of intervention strategies aimed to control *L. monocytogenes* in US retail stores.

P3-43 Survival of *Listeria monocytogenes* and *Clostridium perfringens* in Acidified Pork Lips

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Introduction: Acidification is one way to manufacture shelf-stable food products. Regulatory agencies require validation of this process. Although there are general aspects of these processes which are regulated, the process validation is product specific. A major concern exists with meat processors to comply with USDA's regulations and validate their processes. For this project, pork lips were used.

Purpose: The purpose of this study was to evaluate the survival rate of high counts of *Listeria monocytogenes* and *Clostridium perfringens* inoculated onto pork lips after cooking, followed by acidification at room temperature.

Methods: Pork lips were cooked in water until the internal temperature reached 160°F. The product was allowed to cool down to 100°F and then a mixture of 3 strains of *Listeria monocytogenes* and one of *Clostridium perfringens* were inoculated onto product (9.55 and 7.52 log CFU/g, respectively). After 5 h of holding the product at 100°F, brine solution of pH 3.3 was added to cover the product, and it was capped and stored at room temperature. Tests were done at 5 h after holding at 100°F and then at 24 h, 48 h and 2 weeks. Oxford media was used to detect survival of *Listeria* and TSC for *Clostridium*. The pH of the brined product was measured over time as well.

Results: After 5 hours of holding at 100°F, there were no significant differences in survival of either; after 24-h *Listeria monocytogenes* and *Clostridium perfringens* reduced to 4.73 and 3.38 log CFU/g respectively; after 48 h and 2 weeks both bacteria were reduced to non-detectable levels.

Significance: These data suggest that even with high bacteria counts, after 48 h, there is no survival of either bacterium in this product, and this information can be used in predictive microbiology by USDA as a "worst case" scenario.

P3-44 Measurement of *Listeria monocytogenes* Biofilm Cohesive Energy Using Atomic Force Microscopy

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Introduction: Growth and biofilm formation of *Listeria monocytogenes* in the food processing environment continues to be a major predictor of post-processing contamination of processed foods. Previous work in our laboratory has shown that decreased moisture within biofilms was an indication of increased transfer, which was likely due to decreased cohesive energy between cells and the exopolymeric matrix.

Purpose: The objective of this study was to measure the cohesiveness of *Listeria monocytogenes* biofilms growing using a high loading force rastering method with an atomic force microscope.

Methods: Biofilms were grown on stainless steel in a drip flow bioreactor at 32°C for 72 h and then equilibrated over saturated salt solutions (98%, 75%, 54% or 33% RH) for 48 h at 20°C. Calibrated cantilevers (spring constant and frictional force) were used to image a 5 x 5 µm area of biofilm at a low applied (~0 nN) loading force followed by a series of high applied (100 nN) loading forces in a 2.5 x 2.5 µm sub-area for four subsequent scans followed by a large (5 x 5 µm) low applied force scan. This was repeated for a total of 5 series of high loading force scans. Topography and voltage data were collected from each scan. The changes in topography after each raster treatment series was determined by subtraction.

Results: Raster experiments of biofilms equilibrated at 54%, 75% and 98% RH had very little height reduction, indicating the cohesive energy required to remove biofilm materials were greater than could be put into the system. Raster experiments upon biofilms equilibrated at 33% RH, showed removal of bacterial cells and exopolymeric matrix material. Greater cohesive energy was observed with each high pressure scan (ranging from 13.9 pJ/µm^3 after 4 scans to 78.1 pJ/µm^3 after 20 scans), indicating that the bacteria at the top of the biofilm where more loosely attached than the cells deeper within the biofilm.

Significance: This study found that bacterial cells and exopolymeric substances were more strongly bound in the interior of the biofilm in comparison to the exterior. In addition, moisture appears to be a major factor in the cohesive strength of a biofilm.

P3-45 Prevalence and Molecular Ecology of *Listeria, Salmonella* and Shiga Toxin-producing *Escherichia coli* from Agricultural Environments in Northern Colorado

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Introduction: Listeria monocytogenes, Salmonella and shiga toxin-producing *Escherichia coli* (STEC) cause illnesses which represent a significant public health and economic burden in the U.S. While molecular subtyping is routinely used to characterize human clinical and food isolates, a knowledge gap exists regarding the presence, transmission, and molecular ecology of foodborne pathogens in agricultural environments.

Purpose: The purpose of this study was to probe the molecular ecology of foodborne pathogens in agricultural environments through combined assembly of a collection of natural isolates from grazing pastures and produce farms and molecular subtyping.

Methods: A total of 1,140 soil, 227 drag swab, 525 fecal, and 403 water samples were collected from five pastures and five produce farms in Northern Colorado during sampling trips in the spring, summer, and fall of 2009 and 2010. Each location was divided into four distinct geographic areas and individual samples from each area were pooled prior to microbiological analyses. Samples were pre-enriched using non-selective enrichment procedures and partitioned for subsequent microbiological analysis using modified protocols derived from the U.S. Food and Drug Administration's Bacteriological Analytical Manual to detect each targeted organism. Up to four presumptive colonies representing each target organism from each presumptive positive sample were confirmed using PCR and pathogens were subtyped by pulsed-field gel electrophoresis (PFGE) according to the Centers for Disease and Control PulseNet protocol.

Results: Of the 640 samples analyzed, eight, eleven, and two samples tested positive for *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7, respectively. In addition, twenty samples tested positive for non-O157 STEC (each carrying *stx1* or *stx2*, and/or *eaeA*). Forty-eight samples were positive for other *Listeria* spp., including 20 samples containing unique *Listeria*-like isolates. Phylogenetic analyses of 16s rDNA sequences suggested these isolates represent a new genus closely related to *Listeria*. PFGE indicated eight, eleven, and one unique patterns for *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7, respectively.

Significance: These data suggest the prevalence of foodborne pathogens in Northern Colorado production agriculture environments is low. Subtypes generated by this study molecular will help strengthen epidemiological associations between clinical and agricultural environment isolates.

P3-46 Thermal Inactivation of *Salmonella* and *Listeria monocytogenes* in Model Process Cheese Products

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Introduction: Manufacturers of process cheese products frequently use USDA requirements for commodity process cheese as guidance for pasteurization (>74°C, 165°F for >30 seconds). However, no data are published to confirm the validity of the treatment. Furthermore, fat, pH, and water activity are factors that affect thermal inactivation. Therefore, quantification of thermal inactivation in these types of products is needed.

Purpose: The objective of this study was to validate the thermal inactivation of *Salmonella* and *L. monocytogenes* in model process cheese products.

Methods: Two process cheese products (full-fat: 30% fat, pH 6.0, a_w 0.93; reduced-fat: 10.5% fat, pH 5.8, a_w 0.94) were evaluated. Five-gram portions in boilable pouches were inoculated with approximately 7-log CFU/g of a 5-strain mix of *Salmonella* or *L. monocytogenes*, spread to < 2mm thickness, vacuum sealed, and heated to 82.2, 74 or 65.5°C by submerging in a water bath. Duplicate samples were taken at 0, 15, 30, 45, 60, 90 and 120 seconds at 82.2°C or 74°C or 0, 1, 2, 3, 4, 6, 8, 10 and 12 minutes at 65.5°C and pathogens enumerated on thin agar layer plates using XLD or MOX base with a TSA with overlay. Trials were replicated twice.

Results: L. monocytogenes was more thermotolerant than *Salmonella*, and the full-fat formula afforded greater protection against inactivation than the reduced-fat product. In the full-fat product, *Salmonella* populations decreased >5 log at 0, 15 seconds and 1 minute at 82.2°C, 74°C or 65.5°C, respectively, whereas *L. monocytogenes* populations were reduced by 5 log by 15 seconds, 30 seconds and 6 minutes, respectively. In contrast, >5-log reduction was observed at the first sampling interval for reduced-fat products for both pathogens. The D-value at 65.5°C for *L. monocytogenes* in full-fat was 1.26 minutes but no D-values were calculated for other combinations due to the rapid inactivation at the first sampling interval for each temperature and nonlinear data points.

Significance: This study confirms the protective effect of fat and a_w on thermal inactivation of pathogens and the importance of using thermotolerant organisms when validating thermal inactivation. Data confirms the efficacy of the USDA guidelines for pasteurization of full-fat and reduced-fat process cheese products.

P3-47 Alternative Sigma Factors Affect Biofilm Formation in *Listeria monocytogenes*

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Introduction: Listeria monocytogenes survives under various food-related stress conditions and colonizes surfaces in the form of biofilm. Alternative sigma factor sigB has been implicated in surface attachment and biofilm formation by *L. monocytogenes*.

Purpose: The aim of this research was to study biofilm formation in several isogenic deletion mutants of *L. monocytogenes* lacking alternative sigma factors.

Methods: Overnight cultures of *L. monocytogenes* 10403S and its mutants were diluted in BHI broth until the optical density (OD) of 0.05 at 600 nm. Aliquots of 150 µl were loaded per well in 96-well polystyrene microtiter plates (in 6 replicates) and incubated in SpectraMax 2 with and without agitation at 37°C for 24 h. The growth was recorded by measuring OD at 600 nm. The wells were washed three times with 200 µL phosphate buffered saline (PBS) and each well was stained with 150 µL of 0.5% (w/v) crystal violet solution for 30 min. Plates were washed with 300 µL PBS four times and air dried. Dye bound to adherent cells was resolubilized with 150 µL of 33% (v/v) acetic acid solution, transferred to a micro titer plate and the OD of each well was obtained at 590 nm.

Results: All the cultures grew similarly under the experimental conditions. Agitation increased the biofilm formation of *L. monocytogenes* 10403S and its isogenic mutants, except for Ls 439 (sigH) which showed similar results to those without agitation. Moreover, deletions in the *sigB*, *sigC*, *sigH*, and *sigL* gene reduced the biofilm formation by *L. monocytogenes*. Compared to the wild type, the extent of reduction varied with the mutation and the assay condition.

Significance: The results indicate that several alternative sigma factors including SigB are involved in biofilm formation in *L. monocytogenes*. The results also indicate that growth environment (e.g., with and without agitation) has a significant impact on listerial ability to produce biofilm.

P3-48 Formation and Survival of Stress-induced Filaments by *Listeria monocytogenes*

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Introduction: Listeria monocytogenes often encounters stressful conditions, such as desiccation, during pre-harvest, harvest, processing, and storage. One response to desiccation stress is the formation of filaments, which can exceed 120 µm in length. When conditions improve the filaments form septa and divide into multiple normal-sized cells (1–2 µm). This could impact the observed bacterial load and skew risk assessments.

Purpose: To determine formation, survival, and growth of stress-induced *L. monocytogenes* filaments.

Methods: L. monocytogenes strain Scott A was grown on tryptic soy agar (TSA; a_w 0.99) and TSA with 6% NaCl (a_w 0.96) plates and incubated for 72 hours at 30°C to generate control and filamentous cells, respectively. Cells were tested for growth in fresh tryptic soy broth (TSB) and on TSA, and survival in TSB with sodium nitrite (156 ppm), sodium diacetate/lactate (0.1/1.5% and 0.25/3.75%), or sodium hexametaphosphate (0.1, 0.3, and 0.5%), and on TSA with a rosemary/nisin blend (0.02%). Survival was also determined at 55°C, pH 2, and in a quaternary ammonium or chlorine-based sanitizer. Cell morphology was observed after Gram staining.

Results: L. monocytogenes strain Scott A filaments septated when grown in TSB or on TSA at both 4°C and 30°C. When exposed to the tested antimicrobials, the filaments septated at 30°C and grew to levels comparable to the control cells; at 4°C, no growth was observed in either culture, and a majority of the filaments remained filamented. Control cells and filaments survived equally well at 55°C, while filaments were slightly more tolerant of pH 2 than control cells. The sanitizers were effective in inactivating both cell types.

Significance: The presence of *L. monocytogenes* filamentous cells is significant to food safety because the detection and enumeration of bacterial loads may be underestimated. Information garnered from growth and survival studies will help develop informed recommendations on intervention practices and risk assessments.

P3-49 *Listeria monocytogenes* Sigma B, But Not PrfA, Shows Strain Specific Contributions to Growth under Salt Stress, Including Elongated Lag Phase of a sigB Null Mutant Across Strains

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Introduction: Listeria monocytogenes is well known to have the ability to survive and grow under a variety of stress conditions. Survival and growth under salt and osmotic stress conditions, in particular, appear to be important for certain foods, food associated environments and the intestinal environment of the host.

Purpose: To characterize the role of transcriptional regulators, Sigma B and PrfA, in the survival of *L. monocytogenes* under high salt conditions.

Methods: Three wildtype strains representing *L. monocytogenes* serotype and lineage diversity and their isogenic mutants (Δ*sigB,* Δ*prfA*, and Δ*sigB* Δ*prfA*) were analyzed for the ability to grow in BHI with 11% NaCl at 25°C. Furthermore, to initially explore the mechanism that may contribute to an observed extended lag phase in the Δ*sigB* mutant, microarray analysis was performed to compare transcript levels between the lineage I, serotype 1/2b parent strain and its isogenic Δ*sigB* mutant after 30 minutes exposure of lag phase cells to 11% NaCl at 25°C.

Results: A prolonged lag phase was observed across the three Δ*sigB* mutant strains; however, maximum growth rates were only reduced for the Δ*sigB* mutant of *L. monocytogenes* serotype 1/2a and 4a strains. Interestingly, for the serotype 1/2b strain, the Δ*sigB* mutant reached a higher maximum cell density in comparison to both the parent strain and the Δ*prfA* mutant. Microarray analysis showed lower and higher transcript levels for 136 and 174 genes, respectively, in the parent strain as compared to the Δ*sigB* mutant. Notable genes that showed higher transcript levels in the parent strain include *inlD, groES, sigH, glpK* and *resD*; while, genes that showed lower transcript levels in the parent strain include *dhaK, grpE* and *clpB*.

Significance: These data provide insight into the contribution of Sigma B (ΔB) and PrfA to survival of *L. monocytogenes* under high salt conditions. In particular, the ΔB-dependent transcriptome of *L. monocytogenes* lag phase cells under salt stress was characterized and includes previously identified as well as novel ΔB-dependent genes, including a number of stress response and virulence-associated genes. Acknowledgment: FAPESP (Proc. 2009/14618-0).

P3-50 The Cold Adaptation Phenotypic and Genetic Analysis of *Listeria monocytogenes* Strains Recovered along the Swiss and Canadian Food Supply Chains

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Introduction: Listeria monocytogenes survives and grows on cold-preserved, ready-to-eat foods, leading to significant public health hazards and food safety problems.

Purpose: To examine cold growth phenotypes, molecular genotypes, and cold acclimation gene expression responses associated with *L. monocytogenes* strains colonizing food supply chains in two distinct geographical locations.

Methods: L. monocytogenes strains recovered along food supply chains in Canada and Switzerland were examined. Molecular genotypes were determined using ERIC and REP-PCR based methods. Cold growth kinetics were monitored in BHI cultures held at 4°C based on viable cell counts. Cold adaptation gene expression responses were determined using quantitative real-time qRT-PCR in selected strains that were acclimated to cold stress by incubation (2 h) at 4°C in BHI.

Results: A high level of genetic diversity (29 REP and 4 ERIC PCR patterns) was detected in the two *L. monocytogenes* strain collections from Switzerland and Canada. Strains from both collections grouped into three cold growth categories based on lag phases observed in BHI at 4°C. Group I (GPI) strains adapted quickly (lag-phase < 60 h), Group II (GPII) strains slowly (lag-phase >200 h), and Group III (GPIII) strains displayed intermediate lag phases (60-200 h). Gene expression analysis revealed that fast cold acclimating GPI strains also induced significantly higher *cspA* and *pgpH* gene transcriptional activation compared to slow cold acclimating GPII strains. Sequence analysis of cold adaptation genes, *lmo1078* and *sigL*, coding for a putative UDP-glucose phosphorylase enzyme and an alternative sigma factor σL respectively, revealed strain associated sequence variation, which in some cases leads to truncated non-functional protein products.

Significance: L. monocytogenes strains colonizing food supply chain environments and food products in Canadian and Switzerland exhibit similar cold growth phenotypes. Strain-specific variations in sequenced cold adaptation genes (*sigL* and *lmo1078*) and cold acclimation gene (*cspA* and *pgpH*) expression responses might have implications in cold survival and growth capability of the *L. monocytogenes* strains investigated.

P3-51 Ribotypes of *Listeria monocytogenes* Isolated from Minimally Processed Vegetables in São Paulo, Brazil

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Introduction: Ribotyping is a molecular method extensively applied for identification and typing of bacteria to the strain level. In Brazil, automated ribotyping (AR) has been used mainly for tracking and identifying *Salmonella* and *L. monocytogenes* in foods of animal origin. However, no information on the ribotypes of *L. monocytogenes* present in vegetables in Brazil is available in the literature.

Purpose: The purpose of this study was to examine the occurrence of different molecular subtypes and virulent clones among *L. monocytogenes* strains isolated from minimally processed vegetables (MPV) in São Paulo, Brazil.

Methods: The strains submitted to AR were isolated from watercress (n=1), cabbage (n=2), escarole (n=11), spinach (n=7), mix for yakisoba (n=8), mix for sukiyaki (n=9), collard greens (n=10), lettuce (n=10) and leafy mix (n=12). Among the ribotyped strains, 63 and 7 belonged to serotypes 4b and 1/2b, respectively. Ribotyping was performed using Riboprinter® (DuPont- Qualicon) according to the manufacturer's instructions. EcoRI was used for all isolates and results were automatically compared to patterns stored in the equipment library (DUP-IDs). An isolate was identified when the corresponding *Eco*RI pattern presented a similarity ≥ 0.84 to one of the patterns from DUP-IDs.

Results: Five out of the 70 isolates were not discriminated by the ribotyping method, although they were confirmed as *L. monocytogenes*. A total of 11 ribogroups, with similarity ranging between 0.86 and 0.98 were obtained. These ribogroups were shown to be within 4 different DUP-IDs: 1038, 19191, 18604 e 19175. Most of the strains (66%) belonged to DUP-ID 1038, while 20%, 11% and 0.02% were within DUP-ID 19191, DUP-IDs 18604 and 19175, respectively.

Significance: This study shows the high prevalence (66%) of DUP-ID 1038 among the isolates of *L. monocytogenes* from MPV in Brazil. Our findings are of concern because DUP-ID 1038 is the clonal group of *L. monocytogenes* most frequently associated with listeriosis worldwide.

P3-52 The Role of the Alternative Sigma Factor, SigB, in the Survival of *Listeria monocytogenes* 568 during Desiccation on Stainless Steel

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Introduction: Listeria monocytogenes is a foodborne bacterial pathogen which can colonize the food processing environment thereby leading to cross-contamination. We hypothesized desiccation survival of *L. monocytogenes* is influenced by the alternative sigma factor (SigB) commonly associated with stress response.

Purpose: The objective of this research was to test if the SigB factor contributes to the desiccation survival of *L. monocytogenes* during exposure to different environmental conditions.

Methods: The survival of the parent *L. monocytogenes* 568 *(Lm568)* strain and its Δ*SigB* mutant was compared during desiccation at 43% RH and 15°C on stainless steel. The effect of known osmolytes (proline, betaine and carnitine) in minimal media (MM) with initial NaCl levels of 0.5 and 5% was studied and the inactivation kinetics modeled using the non-linear Weibull model. Transcriptional analysis of osmolyte uptake genes was performed using reverse transcriptase PCR.

Results: Exogenous betaine, carnitine and proline significantly ($P < 0.05$) improved desiccation survival of the Δ*SigB* mutant in the presence of initial NaCl levels of 0.5% and 5.0%. Only exogenous betaine improved ($P < 0.05$) the survival of *Lm568* in 5.0% NaCl. Growth in the presence of each of the osmolytes prior to desiccation enhanced ($P < 0.05$) survival of both strains in MM with 5.0% NaCl, whereas in MM with 0.5% NaCl survival was only improved ($P < 0.05$) for the Δ*SigB* mutant grown in betaine and carnitine. *Lm568* survived desiccation in MM with 5% NaCl significantly better ($P < 0.05$) than its Δ*SigB* mutant. The three osmolytes uptake genes (*gbuA, opuCA, betL*) were transcribed in *Lm568* grown in MM with 0.5 and 5% NaCl. The same genes were transcribed in the mutant with the exception of *opuCA* which was not expressed in MM with 5% NaCl.

Significance: In conclusion, the activities of SigB regulon alone partly explain adaptation to desiccation stress and *SigB*-independent stress genes may be involved in the desiccation stress response.

P3-53 Filamentation Characteristics of Cold-adapted Log Phase *Listeria monocytogenes* under Conditions of Salt and Temperature Stress

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Introduction: Under conditions of stress *L. monocytogenes* can form filaments, strings of non-divided cells that appear as single colonies when enumerated on microbiological media. In foods and food processing facilities, filamentation of *Listeria* could result in underestimation of numbers of cells when challenge studies are conducted to determine if growth occurs.

Purpose: The objective of this study was to determine the degree of filamentation of *L. monocytogenes* at various salt concentrations at 3°C.

Methods: Log phase cells adapted to 15°C of three strains of *L. monocytogenes* were incubated in tryptic soy broth (TSB), 4% NaCl TSB or 8% NaCl TSB at 3°C. Plate counts were done on TS agar and portions of samples were fixed in 1% formaldehyde for flow cytometry and microscopy analysis at regular intervals until cells entered the stationary phase. Flow cytometry results were analyzed to determine the relative cell length of the 10% longest cells in a sample and to determine the percentage of the population of cells that elongated as compared to the control.

Results: Cells grown in TSB filamented during growth with a size ratio from 1–3. When cells were grown in 4% or 8% NaCl, filamentation peaked early in the log phase with cell size increasing 4.8–6.5 times or 14–20 times, respectively, for the longest 10% of the cells in the population. When filamentation peaked, over 55% of the cells in the total population had shifted to an elongated state.

Significance: Results showed that an increase in salt concentration at 3°C increased the degree of filamentation of cells of *L. monocytogenes.* This could potentially lead to an underestimation of numbers of viable *Listeria* when determining cell numbers for validation of food safety.

P3-54 Effects of X-ray Irradiation on *Listeria monocytogenes* and Spoilage Bacteria in Smoked Catfish

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Introduction: Mississippi is the leading state for catfish production in the U.S. Smoked catfish is not yet commercially produced in the U.S.; however, people may smoke catfish at home. Catfish can be contaminated with *Listeria monocytogenes* during smoking or the packaging process due to unhygienic conditions. *L. monocytogenes* can grow well at refrigerated temperatures (5°C) which might make *L. monocytogenes* a threat to the safety of consumers of smoked catfish (listeriosis accounts for approximately 2,500 cases of illness in the United States annually).

Purpose: The objectives of this study were to: 1) Determine if X-ray doses reduce *L. monocytogenes* levels in smoked catfish during storage at 5°C for five weeks and 2) Determine the effect of X-ray doses on the control of the spoilage bacteria on smoked catfish during storage at 5°C for five weeks.

Methods: Smoked catfish fillets (6 samples for each treatment) were inoculated with *L. monocytogenes* (3.9 ± 0.1 log CFU/g) and treated with 0.0, 0.1, 0.5, 1.0 or 2.0 kGy X-ray. Control (non-irradiated, 0.0 kGy) and irradiated samples were then stored at 5°C for five weeks. The population of *L. monocytogenes* was determined at Day 0, 5, 10, 15, 20, 25, 30 and 35. Also, the control (uninoculated-untreated) and uninoculated-treated with the lowest (0.1 kGy) and highest (2.0 kGy) doses were stored at 5°C and tested for mesophiles and psychrotrophs counts during the five weeks of storage. All experiments were replicated three times using two samples per experiment for a total of six data points per treatment. Data were pooled and the mean values and standard deviations were determined. Differences between samples were determined using a Student's t-test, with Microsoft Excel, and were considered to be significant when $P < 0.05$.

Results: The initial *L. monocytogenes* population (3.9 log CFU/g) was significantly ($P < 0.05$) reduced to undetectable level (less than 10 CFU/g) by treatment with 1.0 kGy X-ray. Moreover, treatment with 1.0 kGy kept the population of *L. monocytogenes* under the detectable level until day 30. The initial mesophiles and psychrotrophs counts were significantly reduced from 3.9 and 4.6 CFU/g, respectively, to under the detectable level by treatment with 2.0 kGy X-ray. Treatment with 2.0 kGy kept the mesophiles and psychrotrophs counts significantly lower than the control samples until the end of storage (five weeks).

Significance: The results of this investigation indicated that X-ray at 2.0 kGy is an effective technology to eliminate *L. monocytogenes* and extend the shelf life of smoked catfish.

P3-55 Occurrence and Characterization of Shiga Toxin-producing *Escherichia coli* during Cattle Slaughter for Exporting and Refrigerated Beef Cuts Marketed at the Metropolitan Area of São Paulo

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens implicated as the cause of several human diseases. A variety of foods has been implicated in the outbreaks, but raw or undercooked foods of bovine origin are primarily responsible. STEC are present in the feces of healthy bovines, the main reservoir, and can contaminate meat during slaughter. Brazil is one of the most important beef exporter worldwide and few researches have been done regarding to the presence of STEC in beef carcasses and cuts.

Purpose: The aims of the present study were to determine the occurrence of STEC at three points of cattle slaughter for exporting and in refrigerated beef cuts commercialized in the Metropolitan area of Sao Paulo, Brazil.

Methods: A total of 603 samples were collected from 201 animals at slaughter, three per animal. At retail, 100 refrigerated beef cuts were analyzed. The detection of *E. coli* O157 samples were conducted according to the ISO methodology (16654) and for detection of O26, O103, O111 and O145 serogroups the Surveillance Group for Diseases and Infections of Animals methodology (NRM 006) was used. The isolates were

confirmed as STEC evaluating for the presence of *stx1, stx2, uidA, eaeA, ehxA, rfbO157* and *fliCH7*. Serotyping was done at Institute Adolfo Lutz and genetic diversity was evaluated through PFGE.

Results: Among 201 animals sampled, two (1.0%) were positive for STEC, with seven isolates from hide. The microorganism was not detected in carcasses and half carcasses samples. The seven isolates carried *stx2, uidA, eaeA, ehxA, rfbO157* and *fliCH7* genes, being considered as *E. coli* O157:H7 serotype. None of the isolates produced enterohemolytic activity. PFGE revealed that the seven STEC strains showed two distinct genetic profiles. STEC was not detected from beef cuts marketed at retail.

Significance: These results suggest that, although present in animal hides, the STEC isolation at later stages of food chain was rare, probably due to effective sanitary measures to control cross contamination and transmission of this pathogen along the beef production chain until commercialization. Acknowledgement: FAPESP

P3-56 Survival of *Escherichia coli* O157:H7 in Meat Residues Deposited on the Surface of Meat Packaging Materials

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Introduction: Escherichia coli O157:H7 cells suspended in contaminated food residues, such as meat purge, may potentially be deposited on internal and external surfaces of food packaging materials, where they may attach, survive, and serve as sources of cross-contamination of ready-to-eat foods.

Purpose: This study evaluated survival of *E. coli* O157:H7 in meat residues deposited on four packaging materials commonly associated with storage and display of fresh meat.

Methods: The four packaging materials evaluated were butcher paper, cardboard, PVC overwrap film and vacuum bags. Samples (5×5 cm) were spot-inoculated (0.5 ml) with a ground beef homogenate (10% wt/wt in distilled water) containing an eight-strain mixture of rifampicin-resistant *E. coli* O157:H7 (simulating contaminated meat residues), to achieve target inoculation levels of 4 or 6 log CFU/cm^2. Inoculated packaging materials were stored aerobically in petri dishes at 4 or 25°C for up to 109 days. At regular intervals during storage, samples were analyzed (two repetitions, three samples/treatment/repetition) for surviving total microbial (tryptic soy agar) and inoculated *E. coli* O157:H7 (tryptic soy agar with 100 µg/ml rifampicin) populations.

Results: Populations of meat residue-associated *E. coli* O157:H7 gradually decreased on all packaging materials during storage. However, regardless of inoculation level or storage temperature, pathogen survivors were obtained at the end of storage (109 days) on all tested materials. When inoculated at the low inoculum level (4 log CFU/cm^2), pathogen counts ranged from <0.6 ± 0.8 (cardboard) to 2.1 ± 0.6 (butcher paper) log CFU/cm^2 on materials stored (109 days) at 25°C, and <0.1 ± 0.3 (vacuum pouch material) to <0.3 ± 0.3 (PVC overwrap film) log CFU/cm^2 on samples stored at 4°C. For samples inoculated at 6 log CFU/cm^2, the lowest and highest pathogen counts at 109 days at 25°C were obtained on cardboard (<1.6 ± 1.7 log CFU/cm^2) and butcher paper (2.4 ± 0.5 log CFU/cm^2), respectively. For corresponding samples stored at 4°C, the lowest and highest pathogen counts were obtained on butcher paper (1.0 ± 0.7 log CFU/cm^2) and cardboard (2.2 ± 0.5 log CFU/cm^2), respectively.

Significance: The results indicated that under these conditions, *E. coli* O157:H7 was able to survive for approximately four months on soiled packaging materials. Thus, contaminated meat residues deposited on packaging materials may serve as sources of cross-contamination of foods.

P3-57 Modification of *Escherichia coli* O26:H11 Quorum Sensing mRNA Expression Levels during Growth in Milk in the Presence of *Hafnia alvei* and a Model Cheese Microbial Consortium

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Introduction: Quorum Sensing communication in bacteria (QS) is mediated through the production, the release and detection of small chemical signal molecules.

Purpose: Within the frame of a collaborative project on the risks and benefits of gram-negative bacteria in a cheese model microbial ecosystem, the aim of this work was to evaluate whether *E. coli* O26:H11 could interact with cheese microflora using QS signals.

Methods: Skimmed milk was inoculated with a 10-strain microbial consortium representative of the major groups of microorganisms commonly found in the core of raw milk cheese and with *E. coli* O26:H11 L23A. Sampling was performed after 4h at 33°C and 10, 24 and 48h incubation at 25°C. Cheese consortium was composed of lactic acid bacteria (*Streptococcus thermophilus, Enterococcus faecalis, Lactobacillus plantarum, Lactococcus lactis, Leuconostoc mesenteroides*) and technological flora (*Debaryomyces hansenii, Yarrowia lipolytica, Arthrobacter arilaitensis, Corynebacterium casei, Staphylococcus equorum*). Population and gene expression were quantified for mixed cultures with and without *H. alvei* B16. Microbial population quantifications were done by enumeration on specific agar media. (RT)-Q-PCR quantifications of *E. coli* were performed targeting genes involved in QS (*LuxS, Pfs*), metabolic activity (16S rRNA, *tuf, GAPD*) and stress response (*UspA, GroEL, RpoS*). While (RT)-Q-PCR quantifications of *H. alvei* were performed targeting *tuf* and *LuxS* genes. All experiments were carried out for 3 independent mixed cultures.

Results: Without *H. alvei*, targeted *E. coli* O26:H11 expression levels mainly correspond to metabolic and to signals potentially involved in QS activities. Similar expression levels are observed for *LuxS, Pfs* and *tuf* genes underlining the correlation and importance of QS related genes expression during growth. Maximal *E. coli* population reaches 7.83 log and *RpoS* expression quantification is possible after 4 hours. In the presence of *H. alvei*, *E. coli* growth is strongly inhibited and remains at 5.56 log. Unlike previously observed, *E. coli LuxS* and *Pfs* transcripts are largely higher than *tuf* transcripts. While expression levels recorded for *H. alvei* clearly show a predominant metabolic activity with low *luxS* expression.

Significance: This study reports modifications of mRNA expression levels of *E. coli* O26:H11 in the cheese model in the presence of *H. alvei*, underlining the incidence of signals potentially involved in QS when interacting with complex ecosystems.

P3-58 Detection of Shiga Toxin-producing *Escherichia coli* in Ground Beef Sold in São Paulo, Brazil

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Introduction: Although *E. coli* O157:H7 is considered the main Shiga toxin-producing serotype (STEC) involved in outbreaks of foodborne illnesses, other serotypes are involved worldwide, such as O103, O111, O145 and O26. STEC are responsible for symptoms ranging from mild diarrhea to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, which can lead to death. These strains present several virulence factors such as, the production of Shiga toxin (*Stx*) and intimin, a protein involved in the attachment to the cell and haemolysin. Despite the importance of ground beef as a vehicle of STEC, little is known about its presence in the meat sold in São Paulo, Brazil.

Purpose: The aim of this study is to investigate the presence of STEC in ground beef at retail level in São Paulo, Brazil, and to characterize the virulence factors *stx*$_1$, *stx*$_2$, *eae* and *ehx*, as well as, to identify *E. coli* O157:H7 isolates using the genes *uid*, *rfb*$_{O157}$ and *fliC*$_{H7}$.

Methods: Ninety-six samples were acquired at different districts of São Paulo. Microbiological tests for the detection of STEC were performed following the ISO 16654 methodology for the detection of *E. coli* O157 serogroup using sorbitol MacConkey agar, sorbitol MacConkey agar plus potassium tellurite and cefixime and chromogenic agar as isolation media. The detection of serogroups O103, O111, O145 and O26 was performed according to the methodology described by the Surveillance Group for Diseases and Infections of Animals (NRM 006) using MacConkey agar. Suspected colonies were evaluated for the presence of target genes.

Results: From the total of 1025 isolates obtained, 1 (0.5%) was positive for stx_1, none (0%) were positive for stx_2, *eae* and *ehx*, 6 (3.1%) were positive for *uid*, 30 (15.7%) were positive for $fliC_{H7}$ and 12 (6.3%) for rfb_{O157}.

Significance: This study reports, for the first time, the presence of STEC in ground beef acquired at retail level in São Paulo. Therefore, the ingestion of undercooked ground beef can be a risk to the consumers. Acknowledgment: FAPESP.

P3-59 Thermal Tolerance of O157 and Non-O157 Shiga Toxin-producing *Escherichia coli* Strains in a Broth System

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Introduction: Recent foodborne illness outbreaks in the United States linked to non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have highlighted the importance of these pathogens in human disease. There are six non-O157 serogroups most often associated with human illness: O26, O45, O103, O111, O121 and O145.

Purpose: In this study, we compared the thermal tolerance in broth of a representative strain from each of the six serogroups with a reference O157:H7 STEC strain (ATCC 43895).

Methods: Each strain was grown in brain-heart infusion broth (BHIB; pH 7.0) to stationary phase (~10^9 CFU/ml), and 0.2 or 2.0 ml of stationary-phase culture was used to inoculate 18 ml BHIB pre-warmed in a shaking water bath for thermotolerance experiments at 54.4°, 60.0° or 65.5°C. Samples were heated for up to 120 min at 54.4°C, 4 min at 60.0°C, or 45 sec at 65.5°C, with periodic sampling (3 trials per strain). Heated aliquots (1 ml) were immediately transferred to pre-chilled Butterfield's Phosphate Diluent (BPD). Surviving inocula were enumerated after serial dilution in BPD and plating on modified Eosin Methylene Blue agar (37°C, 24 h). For each strain/temperature, $\log_{10}$CFU/ml was plotted and D-values determined from the linear portion of the curve using Excel software. D-values were compared across all strains at each temperature using analysis of variance.

Results: D-values ranged from 9.39 to 31.15 min at 54.4°C, 0.63 to 1.28 min at 60.0°C, and 0.08 to 0.21 min at 65.5°C. At 54.4°C, STEC O103:H2 and O157:H7 were significantly more thermal-tolerant (had a higher D-value) than the rest of the strains ($P < 0.05$). At 65.5°C, thermotolerance of STEC O45:H2 was not significantly different from O157:H7 ($P \geq 0.05$). At 60.0°C, O157:H7 was significantly more heat tolerant than five other STEC evaluated ($P < 0.05$) but not significantly different from 0121:H19 ($P \geq 0.05$).

Significance: These results suggest that thermal-processing intervention treatments that target destruction of *E. coli* O157:H7 may have adequate lethality against other STEC. Further studies in beef tissues as well as challenge studies involving actual beef products are warranted.

P3-60 Evaluation of a New Rapid Screening of *Pseudomonas aeruginosa* in Drinking Water Using Flow Cytometry Detection

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Introduction: Pseudomonas aeruginosa is an environmental ubiquitous water organism and is one of the mandatory pathogens that has to be tested in drinking water filling lines. Standard agar-based methods require 5 days to get final results.

Purpose: To reduce the time-to-release of drinking water in accordance with *P. aeruginosa* criteria, a new enrichment broth combined with flow cytometry detection was developed.

Methods: A new enrichment broth was developed in accordance with Flow Cytometry Method (FCM) requirements (i.e., compatibility background) and specificity criteria needed for screening detection after 24 hours incubation at 30°C. This qualitative method was evaluated on different criteria: limit of detection, specificity, accuracy and precision. Inclusivity evaluation was performed on 7 isolates of *P. aeruginosa* (ATTC and wild strains) to demonstrate the capability of the new method to detect low levels of *P. aeruginosa* (<1CFU/250ml of drinking water). Exclusivity was performed on several non-*P. aeruginosa* bacterial species including *Pseudomonas* spp., Gram-negative and Gram-positive strains (12 species). The Spearman Karber test was applied to evaluate LOD50 value. Accuracy and precision of FCM method was compared to traditional method using Ki2 test.

Results: There were no statistically significant differences in the detection of *P. aeruginosa* using a traditional method versus FCM. LOD50 of *P. aeruginosa* with FCM method was calculated and confirmed to be at least as good as the traditional method. Artificial contamination and natural contamination were detected and correlated with the conventional method as well.

Significance: Flow cytometry for *P. aeruginosa* screening detection can be applied in drinking water filling lines to release products within 24 hours, instead of the 5 days needed with a conventional method. A specific enrichment broth to assure specificity is required.

P3-61 Inactivation of Shiga Toxin-producing *Escherichia coli* in Single Strength Lemon and Lime Juices

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Introduction: According to the CDC, the most commonly reported serotypes are O26, O45, O103, O111, O145 and O121. There are many indications that the Food Safety and Inspection Service (FSIS) intends to declare the above mentioned non-O157 STEC serotypes as adulterants in products such as ground beef.

Purpose: The objective of this study was to determine the inactivation of non-O157 Shiga toxin-producing *E. coli* (STEC) serotypes in comparison to O157 in commercially-produced shelf-stable lemon and lime juices containing preservatives.

Methods: A challenge test was conducted for stationary-phase cells of *E. coli* O157:H7 and six non-O157 STEC serotypes (O26, O45, O103, O111, O145 and O121) in commercially produced shelf-stable single-strength lemon and lime juices containing preservatives. The juice bottles were inoculated with a three-strain composite of each individual serotype to give a final inoculum level of approximately 10^6 CFU/ml. After 72 h of storage at 22 ± 1°C, 10 ml of juice sample was drawn from each bottle and transferred into 250-ml Erlenmeyer flasks containing 87 ml TSB and 2.7 ml of sterile 3N NaOH to reach a final pH of 7.0 ± 0.1. Further decimal dilutions were made by transferring 1 ml of the sample into 9.0 ml of TSB (pH 7.0). A loopful of enrichment broth from turbid flasks/tubes was streaked onto Rainbow agar to confirm the recovery of STEC.

Results: Similar storage conditions were applied to inoculated juice products as previously published by our laboratories. The only difference was that in addition to the challenge study using O157:H7, products were inoculated with a three-strain composite of STEC O26, O45, O103,

O111, O121, and O145. No STEC survivors were detected in either lemon or lime juice stored for 72 h at room temperature (22 ± 1°C) in any of the validation trials. Therefore, the incubation conditions used in the present validation test provided a greater than 6-log reduction of STEC strains tested in commercial shelf-stable single-strength lemon and lime juices, with preservatives.

Significance: Our validation test confirmed the results previously reported indicating the storage of single-strength lemon and lime juices for 72 h at 22°C as an alternative to pasteurization, to meet the FDA 5-log pathogen reduction performance standard. Therefore the interventions applied to inactivate *E. coli* O157:H7 in lemon and lime juices stored in the above mentioned conditions would similarly inactivate the six non-O157 STEC serotypes.

P3-62 Illustration of the Diversity in Microbial Resistance to Pulsed Electric Fields in Beer Using Surface Response Modeling

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Introduction: Response surface modeling (RSM) is increasingly applied to optimize microbial inactivation by pulsed electric fields (PEF), where the inactivation occurs as a result of a combination of variables.

Purpose: This study was conducted to evaluate the best RSM function for the description and comparison of microbial resistances to PEF while considering the variation in treatment conditions.

Methods: Surviving population datasets of four microorganisms (*Bacillus subtilis*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Salmonella choleraesuis*) in beer exposed to two PEF processing inlet temperatures (3.7 ± 0.4 or 13.6 ± 0.8°C) were analyzed using the "RSM" package from the R software environment for statistical computing, aided by the R Commander package. Three function types (first order, first order plus interactions, and complete quadratic) were analyzed for their goodness-of-fit when describing the surviving population (S: 0 to 8.8 log CFU/mL) as a result of the combination of electric field (E: 3.5 to 4.5 kV/mm) and treatment time (t: 0.4 to 2.3 ms), or applied specific energy (Q: 0.5 to 2.3 MJ) and temperature rise (ΔT: 17.8 to 54.0°C).

Results: The resulting S from the E and t combination analysis was better described using the first order plus interactions resulting in Akaike Information Coefficients (AIC) ranging from 8.7 to 80.5. The gram negative *S. choleraesuis* showed slightly more resistance at the highest initial processing temperature. The relation between Q and ΔT affecting S was better described using a first order function (AIC's between 17.4 and 73.4).

Significance: Mechanistic and probabilistic models have been used to describe microbial inactivation trends. However, empirical models like RSM need to be evaluated for their application to assess processing conditions in multivariate processes like PEF.

P3-63 A Monitoring of Norovirus in Ground Water of Kangwon-do, Korea, from Years 2009–2010

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Introduction: Norovirus(NoV) is commonly associated with human sewage and is responsible for numerous cases of waterborne and foodborne gastroenteritis every year in Korea. Outbreaks of acute gastroenteritis caused by contaminated groundwater have been reported.

Purpose: The purpose of this study is to prevent NoV-associated foodborne gastroenteritis in an early stage by monitoring the presence of NoV in ground water used for food processing at school facilities.

Methods: NoV was analyzed by One-step RT-PCR and seminested-PCR assays of ground water in Kangwon-do, Korea, from 2009-2010. In this survey, sampling was carried out twice in 6-month intervals. A total of 136 samples were collected from 34 schools, which included 21 elementary schools, 7 middle schools and 6 high schools.

Results: NoV was not detected in all 136 samples, demonstrating that the underground water of Kangwon-do was not contaminated by NoV. This result was contrasted with the contamination rate 3.2% (62 cases/ total 1,964 cases) in 2009 and 0.7% (15 cases/2,225 cases) in 2010 identified for the other regions excluding the Kangwon-do region.

Significance: It appears that the underground water of Kangwon-do is more suitable for drinking water.

P3-64 Detection and Characterization of Norovirus in Drinking and Reclaimed Water Implicated in a Gastroenteritis Outbreak after the Chilean Earthquake

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Introduction: After a major earthquake in south-Chile, the city of Antofagasta in the North suffered its largest recorded gastroenteritis outbreak in March, 2010. It soon became a rapidly spreading epidemic with a large number of cases. The cause of this coincidence was the interrupted supply of chlorine produced by a southern company, which resulted in the lack of properly chlorinated water.

Purpose: The Chilean authorities requested technical assistance from the US-FDA for the norovirus detection. The objective of this project was to detect and characterize enteric viruses from contaminated drinking and reclaimed water from different sources in Antofagasta.

Methods: Several water stations were identified using the epidemiological data provided by the SEREMI-Antofagasta office. From each station, both influent and effluent samples were collected from its water treatment plant (WTP), desalinization water treatment plant (DWTP), and a reclaimed waste water treatment plant (WWTP). Forty liters of each water sample were concentrated by tangential flow filtration using 30 KDa polysulfone membrane filter. Multiplex RTqPCR for norovirus GI (NoV GI) and norovirus GII (NoV GII) was carried-out using Qiagen One step RT-PCR kit. NoV amplicons were sequenced using the ABIS Prism 3130 and compared to those in GenBank.

Results: NoV GII was detected in influent samples collected from WTP, DWTP, and WWTP. NoV GI was not detected in any water samples. Effluent water samples analyzed from WTP and DWTP were negative, indicating the effectiveness of the water treatment plants in Antofagasta city after the earthquake. However, the effluent water sample from the WWTP was positive for NoV GII suggesting a possible source of contamination since this water was used for irrigation purposes. Norovirus GII sequences from DWTP and WTP isolates were not related to the clinical isolates, but isolates positive for GII from the WWTP influent and effluent showed 96% identity with some of the clinical isolates.

Significance: This was the first analytical study in which viruses implicated in an outbreak were linked to environmental waters sources in Chile. The results confirmed the epidemiological findings of the Health Ministry, which implied that ineffective chlorine treatment of reclaimed waste water leads to the exposure of produce to NoV contamination.

P3-65 Do Beliefs and Knowledge of Children's Caregivers Predict Safe Food Handling Practices?

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Introduction: Children have a higher risk for foodborne illness compared to adults due to their underdeveloped immune system, lower body weight and lack of control over meal preparation. Caregivers responsible for food preparation directly affect the food safety risk for children.

Purpose: To determine whether beliefs and knowledge of caregivers of children (<10 years) predict safe food handling practices.

Methods: Four surveys were developed based on FightBac!™ concepts and Health Belief Model (HBM) constructs. Each survey provided knowledge and practice questions related to one FightBac!™ concept: Clean, Chill, Cook or Separate. HBM construct questions (related to foodborne illness) and demographic questions were the same for all surveys. A statistical model was established to determine relationships between knowledge, practice and HBM constructs. Questions were tested prior to survey delivery (Lum, 2010). Each survey was sent to a nationwide random sample of 750 households with children. Descriptive statistics and inferential statistics using multiple regression analysis were used.

Results: Respondents were predominately female (68%), Caucasian (78%), 30-49 years (82%) with 1–2 children (81%). Knowledge was a significant predictor for safe food handling within the Chill concept relating to proper food storage (b = 0.19, $P = 0.037$), Cook concept (b = 0.22, $P = 0.023$) and Separate concept (b = 0.19, $P = 0.025$) in our statistical model. High perceived susceptibility (b = 0.20, $P = 0.017$) and high perceived severity (b = 0.19, $P = 0.050$) significantly predicted safe food handling within the Separate and Clean concepts, respectively. Self-efficacy significantly predicted safe food handling in the Separate concept (b = 0.21, $P = 0.013$), and in the Clean concept (b = 0.32, $P = 0.001$). High cues to action significantly predicted safe food handling (b = 0.24, $P = 0.014$) within the Clean concept.

Significance: Knowledge is the strongest predictor of safe food handling practices, accounting for 19–22% of variability in Chill, Cook and Separate, followed by cues to action (24%), perceived susceptibility (20%) and perceived severity (19%).

P3-66 The Prevalence of Microwave Cooking in 60 Minnesota Food Service Establishments — An EHS-Net Survey

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Introduction: Microwave cooking of prepared but not ready-to-eat food products by consumers has been a documented risk factor in several foodborne disease outbreaks, including the 2007 multi-state frozen pot pie outbreak, which resulted in 401 *Salmonella* cases. Although there have been several outbreaks related to consumer microwave cooking, the extent of microwave oven use in restaurants and other food service establishments was not well documented.

Purpose: The aim of this study was to better understand microwave usage in food service establishments by obtaining preliminary data on the prevalence of usage, the types of foods heated in microwave ovens, and microwaving protocols.

Methods: From 2008–2009, data were collected on a convenience sample of 60 food establishments within one area of Minnesota. A study questionnaire was administered by Minnesota EHS-Net environmental health specialists to establishment managers.

Results: Facility types involved in the study were: Fast food restaurant (12), sit down restaurant (33), restaurant w/in grocery (1), school foodservice (6), nursing home (1), hotel/motel (6), and daycare (1); 25 of the 60 establishments classified their food preparation as complex. The number of microwaves varied, with 60% of the establishments having 1 microwave, 27% having 2 microwaves, and 13% having 3 or more. The majority of establishments reported using microwaves for warming up commercial RTE products (66%) such as bakery items and cheese sauce, and for warming up foods such as soups and chili for palatability (50%). Establishments also reported using microwaves for thawing foods (22%) such as meat or poultry. No establishments reported using the microwave to cook raw meats, poultry, or eggs, and 93% of the establishments reported that their employees received training for cooking specific foods in the microwave.

Significance: Until now, there has been little information regarding microwave usage in food service establishments. This survey indicates that food establishments are knowledgeable about proper microwave usage and use microwaves mainly for heating rather than cooking. This suggests the risk of foodborne illness from improper microwave cooking may be minimal in these establishments.

P3-67 Effect of Covering on the Cooling of Prepared Foods

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Introduction: Common practice for the storage of prepared food in commercial kitchens is to transfer the food into a pan and cover it with a flexible wrap before it is placed into a cooling unit. The FDA Food Code stipulates that such food be cooled from 57.2°C to 21.1°C within 2 hours and from 57.2°C to 5.0°C within a total of 6 hours. Experimental data were generated, showing that with a cover and in a well-running cooler, the stipulations are rarely met. Finite element software (COMSOL) was used to study heat transfer under these conditions and showed that, as expected, the headspace air acts as an insulator, preventing efficient cooling of food. Yet, removing the cover presents problems with hygiene and safety.

Purpose: Patting the cover to contact the food, thereby eliminating the headspace, is a potential solution. Comsol was used to determine the degree to which cooling could theoretically be improved by this action. It was also used to determine to what extent imperfect patting, i.e. leaving headspace near the wall, could be tolerated.

Methods: A Comsol model was developed to describe the heat transfer to a pan of cooling food. The effect of pan height (6.35 to 10.16 cm), food thickness (5.08 and 7.62 cm), cooling temperature (-26.1, -20.6 and 3.9°C), and cooling environment (non-convective and convective cooling) was examined. The model was also developed to allow various amounts of headspace to remain.

Results: The results for the worst case (non-convective refrigeration at the greatest headspace height) showed that differences between actual and the Food Code stipulated temperatures were improved by 11.5% (for the 21.1°C endpoint) and 21.9% (for the 5.0°C endpoint). Regardless, the Food Code stipulations still could not be met regardless of cooling temperature. In convective cooling, patting the cover to contact the food did allow the Food Code cooling requirements to be met. It was also found that imperfect patting, i.e., leaving headspace near the wall, could be tolerated up to 6.35cm from the wall at the lowest convection rate examined without jeopardizing Food Code requirements.

Significance: Although the headspace slows down cooling in pans of foods, its elimination does not enhance cooling sufficiently to meet the FDA Food Code requirements in non-convective refrigeration. Eliminating the headspace of pans subject to convective cooling does accomplish this to the point where imperfect contact can be tolerated.

P3-68 Effect of a Non-destructive High Voltage Leak Detection (HVLD) Technique on Physical Properties of Plastic- and Foil-laminated Packages for Foods

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Introduction: High-voltage leak detection (HVLD) is a technology that has successfully been used to test the integrity of hermetically sealed flexible and semi-rigid packages in industry. There is, however, a concern that a package may be damaged due to high voltages applied during the inspection.

Purpose: This research was conducted to evaluate the effect of high voltages on package integrity including oxygen transmission rate (OTR), water vapor transmission rate (WVTR), and mechanical properties for plastic- and foil-laminated films.

Methods: Pouches containing 200 ml of 1% NaCl solution were prepared from plastic- and foil-laminated films without and with pinholes (10 µm in diameter). While high voltages ranging from 0.5-10kV were applied to a sample pouch through a support electrode, the surface of the pouch was scanned with an inspection electrode. The OTR and WVTR of sample pouches without pinholes were measured before and after high voltage treatment. Pouches with pinholes were exposed to high voltages and monitored for change in pinhole size by using a microscope. A minimum voltage required to detect delamination in foil-laminated packages was determined.

Results: After inspection at a highest voltage level of 10kV, the OTR and WVTR of foil-laminated pouches increased by 34% and 31%, respectively, whereas there were no significant changes in both OTR and WVTR values for plastic-laminated pouches ($P > 0.05$). Microscopic analysis of pinholes showed that the diameter of pinholes in plastic-laminated pouches changed from 9.5 + 0.6 to 18.7 + 0.5 and 44 + 0.2 µm after high voltage treatment at 0.8 and 9.9 kV, respectively. For foil-laminated pouches, the size of a 10µm pinhole increased to 17 + 0.7 and 34 + 0.5 µm after the inspection at 0.5 and 4.4 kV, respectively. Surface delamination in foil-laminated pouches was observed when the applied voltage was greater than 3.5kV.

Significance: These results suggest that HVLD technique is a promising technique that can be safely applied to detect pinhole defects in plastic packages.

P3-69 Sensitivity of Lactic Acid Bacteria as a Biomarker to Detect Rodenticides in Milk

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Introduction: High accessibility to dairy farms and poor levels of biosecurity creates the possibility of intentional contamination of milk with toxins, which could lead to potentially devastating effects on human health. Current techniques to detect toxins in milk tend to be expensive and time consuming. Thus, there is an urgent need for developing simple on-farm techniques that can detect toxins in raw milk.

Purpose: The objective of this study was to determine if lactic acid bacteria (LAB) could be applied as a biomarker to detect the presence of rodenticides in milk.

Methods: Serially diluted rodenticides (brodifacoum, bromadiolone and diphacinone) were added to tubes containing MRS broth. Commercial yogurt culture was then inoculated in MRS broth sample and incubated at 42°C for 6 hours. The Optical Density (610 nm) and pH of the broth was recorded at 2, 4, and 6 hour intervals. The lactic acid production, bacterial counts and alpha and beta galactosidase activity also were determined at the end of incubation.

Results: The yogurt cultures showed detectable sensitivity (significant change ($P < 0.05$) in Optical Density in the medium) to diphacinone at 0.005 mg/ml to brodifacoum at 0.01mg/ml, and to bromadiolone at 0.04 mg/ml. There also were statistically significant differences ($P < .05$) in the bacterial count, pH and lactic acid production in the presence verses absence of toxins in the medium. The results indicate that yogurt culture could be used as a biomarker for the early detection of rodenticides in milk.

Significance: This system can be improved to a highly sensitive, environmentally safe, quick and accurate test for the presence of pesticides in dairy food products.

P3-70 Determination of Histamine in Mahi-Mahi Fillets (*Coryphaena hippurus)* Implicated in a Foodborne Poisoning

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Introduction: An incident of foodborne poisoning causing illness in 53 victims due to ingestion of fish fillets occurred in January, 2009 in Kaohsiung city, southern Taiwan.

Purpose: The infected population suffered from allergy-like symptoms, including rash, nausea, headache, itching and flushing, but all recovered within 12 hours.

Methods: To elucidate the causative agent, two suspected fish fillets were collected from the suspected restaurant and analyzed for the levels of biogenic amine, total coliform, *E. coli*, total volatile basic nitrogen and histamine-forming bacteria.

Results: Based on the finding that high contents of histamine (>30 mg/100 g) were detected in the suspected mahi-mahi samples, we speculate the temperature abuse of the fillets before cooking contributed to the presence of high histamine levels in the mahi-mahi fillets and resulted in foodborne poisoning. Although histamine-producing strains, *Bacillus subtilis* and *Enterobacter aerogenes* were isolated from suspected fish samples, they might not be the main contributors to histamine accumulation because of low histamine production.

Significance: These results re-emphasize proper handling temperatures for seafood and offer important awareness that *Coryphaena hippurus* fillets could become a hazardous food item in causing histamine poisoning, even though no quality deficiency was observed on the fillets.

P3-71 Bacteriological Quality and Histamine-forming Bacteria Associated with Fish Meats and Environments in Fish Processing Factories

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Introduction: The primary concern of the HACCP system is proper handling and processing of potentially hazardous fish to reduce growth of histamine-producing bacteria capable of supporting histamine production.

Purpose: This research was undertaken by testing fish meat and environmental surface samples obtained from HACCP and non-HACCP fish processing factories in Pingtung, Southern Taiwan, to understand their hygienic quality and histamine-forming bacteria prevalence.

Methods: This study determined the bacteriological and hygienic quality of 20 fish samples and 21 environmental surface samples obtained from three fish processing factories.

Results: It was showed that the HACCP factory was more hygienic than non-HACCP factory in Taiwan. Although the histamine content in all fish samples was less than the 5 mg/100 g U.S. FDA guideline value, 4 out of 15 histamine-producing isolates isolated from the fish and environmental surfaces were identified to be prolific histamine-formers with the ability to produce >185 ppm histamine in TSBH medium.

Significance: Prolific histamine-producing isolates isolated from fish skin, floor and fish meat samples demonstrate the potential risk for contamination of fish with these bacteria and greater risk of histamine development under mild to high temperatures and moderate to long exposure times.

P3-72 Prevalence of Histamine in Scombroid Fish and of Histamine Poisoning in Brazil from 2007–2009

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Introduction: Histamine in fish has been associated with poor hygienic and sanitary conditions during ante- and post-capture handling and high holding temperatures. There is little information regarding histamine and food intoxication in Brazil.

Purpose: The objectives of this study were to investigate the quality of scombroid fish with respect to histamine and to investigate the occurrence of histamine poisoning in Brazil.

Methods: Samples of Scombroid fish were obtained during this period and analyzed for histamine by ion-pair high performance liquid chromatography with fluorimetric detection after post-column derivatization with o-phthaladehyde. The samples were captured by means of long line and troll caught.

Results: The fish mostly captured were yellowfin (*Thunnus albacares*), skipjack (*Katsuwonus pelamis*) and swordfish (*Xiphias gladius*). The prevalent conditions during capture by long line were 6 to 107 days of fishing; water surface temperature - 18.3 to 30.8°C; depth of the bait 800 to 5500 m; number of lines –880 to 2200; fishing area - latitude 04°47'N to 22°20'S and longitude 40°15'W to 50°22'W; and 3,320 to 195,000 kg of captured fish. The prevalent conditions during capture by troll caught were 2 to 35 days of fishing; water surface temperature - 22.8 a 27.6°C; depth of the bait –100 to 2000 m; fishing area - latitude 22°40'S to 32°44'S and longitude 20°11'W to 48°30'W; and 3,669 to 123,823 kg of fish captured. After capture, the fish were bled, eviscerated and the flippers, head and tail were cut. Among the 864 samples analyzed, only 7.3% contained histamine at levels varying from non detected (<0.56 mg/kg) to 878.22 mg/kg (mean 5.8 mg/kg and median of 0.0 mg/kg). Only two of the 96 lots analyzed did not attend the European legislation. The levels of histamine detected were affected by the year, month of the year and region of capture.

Significance: Histamine in fish has been associated with poor hygienic and sanitary conditions during ante- and post-capture handling and high holding temperatures. There is little information regarding histamine and food intoxication in Brazil.

P3-73 Histamine Accumulation and Histamine-forming Bacteria in Dried Smooth-tailed Trevally (*Selariodes leptolepis)* Products

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Introduction: The safety determination of 27 dried smooth-tailed trevally (*Selariodes leptolepis*) products sold in Taiwan during 2008–2010 showed that histamine contents in most of the dried smooth-tailed trevally products (48.10%, 13/27) exceed the 5 mg/100 g U.S. FDA guideline value. Consumption of these products could lead to scombroid poisoning in consumers. The *Enterobacter aerogenes* isolate was proven to be a prolific histamine former.

Purpose: To prevent histamine poisoning due to dried smooth-tailed trevally products and improve the quality of these products.

Methods: Our study examined the optimal condition of growth and histamine formation for *E. aerogenes*, which was isolated from dried smooth-tailed trevally products.

Results: The levels of pH, salt content, water content, water activity (a_w), total volatile basic nitrogen (TVBN), aerobic plate count (APC), total coliform (TC) and *Escherichia coli* in 27 dried smooth-tailed trevally products ranged from 5.82 to 6.64, 1.43% to 20.2%, 13.49% to 54.48%, 0.59 to 0.78, 10.79 to 88.92 mg/100 g, <1 to 7.30 log CFU/g, <3 to >1100 MPN/g and <3 to 460 MPN/g, respectively. *E. aerogenes* was studied for its growth and ability to promote the formation of TVBN and histamine in *S. leptolepis* added to various NaCl concentrations at 25°C. The bacterial number reached 10 log CFU/g in 24 h with 0% and 5% salt content. The highest TVBN values were 76.27 and 77.07 mg/100 g after 48 h storage for added to 0% and 5% salt content, respectively. The VBN values and histamine content of 15% and 20% groups were elevated slower during 48 hr storage.

Significance: The results revealed that sun-drying of salted smooth-tailed trevally with 0-10% NaCl enhanced the growth of *E. aerogenes* and thus accelerated toxic amine formation.

P3-74 Effects of Storage Temperature on Accumulation of Histamine in Tuna Products

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Introduction: There have been several reports in the past decade of histamine (Hst) poisoning associated with tuna salad products. Contamination of retorted tuna could result from the introduction of Hst-producing bacteria (HPB) from the processing environment or by addition of raw ingredients. There are limited data on effects of temperature abuse on Hst accumulation in these products.

Purpose: The objective of this study was to determine effects of storage temperature on growth of HPB and Hst accumulation in uninoculated and inoculated plain tuna and tuna salad products and to ascertain if a food safety hazard exists.

Methods: Twenty-five g samples of plain retorted tuna and tuna salad (tuna with mayonnaise) were inoculated with high- or low-HPB and incubated at 10°C (7 d), 18°C (6 d), or 25°C (3 d). Uninoculated samples were used as negative controls. Numbers ($\log_{10}$CFU/g) of HPB were determined by standard plate count on 1% tryptone:2% NaCl agar at 25°C. Numbers of total and species-specific HPB were determined by MPN real-time PCR ($\log_{10}$MPN/g). Hst (ppm) was determined per AOAC 977.13.

Results: HPB increased to 7.3-8.2 $\log_{10}$CFU/g (8.4-8.7 $\log_{10}$MPN/g) in plain tuna and to 6.5-7.4 $\log_{10}$CFU (6.6-8.3 $\log_{10}$MPN/g) in tuna salad after one day at 25°C. High-HPB produced 635 to 3722 ppm Hst, while low-HPB produced 12 to 31 ppm Hst. Three days of temperature abuse at 18°C were required to produce 8 $\log_{10}$CFU and MPN/g and 2848 ppm Hst. Storage at 10°C for 7 days prevented growth and Hst production by HPB. No HPB were detected in uninoculated samples.

Significance: Storage at 10°C of plain tuna and tuna prepared with mayonnaise prevented growth of HPB and production of unacceptable levels (>50 ppm) of Hst. Adherence to the retail food code (storage at 4°C) will prevent Hst poisoning associated with temperature abuse of tuna salad products and guarantee food safety.

P3-75 Isolation of *Salmonella* spp. and *Vibrio* spp. from Seafood Sold in Singapore

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Introduction: Foodborne hazards in seafood have only recently received increased attention in Singapore since the Indian rojak (a traditional fruits and vegetable salad) outbreak in 2009. *Vibrio* spp. are usually the bacteriological agent of primary concern in seafood because of its wide distribution in the marine environment. *Salmonella* spp. are also frequently isolated from seafood, indicating cross-contamination. Thus, it is important to understand the microbiological quality of seafood for assurance of food safety.

Purpose: The aim of this study was to evaluate microbiological quality and to determine the prevalence of *Listeria* monocytogenes, *Salmonella* spp. and *Vibrio* spp. in seafood sold in Singapore.

Methods: One hundred five (43 prawn, 30 shellfish and 32 fishball) samples were collected from major supermarkets and wet markets located around Singapore. Aerobic and psychrotrophic bacterial counts were performed. For the isolation of *Listeria*, *Salmonella* spp. and *Vibrio* spp., the samples were enriched in the appropriate media, followed by streaking onto selective and chromogenic agars. Presumptive-positive colonies were then subjected to API and VITEK 2 for further identification. Confirmed *Salmonella* spp. were tested for serotyping and *Vibrio* spp. were tested for antibiotic resistance using the disk-diffusion assay.

Results: The aerobic and psychrotrophic bacterial counts for prawn, shellfish and fishball were in the range of 4–5 log CFU/g in a 25-g sample. No *Listeria monocytogenes* was isolated from the samples. One *Salmonella* Lexington was isolated from a thawed frozen white shell meat product and two *Vibrio parahaemolyticus* strains were isolated from commercial fishball and cooked shrimp meat products. Both strains of *V. parahaemolyticus* were resistant to ampicillin but sensitive to streptomycin, chloramphenicol, tetracycline and erythromycin.

Significance: The results show that seafood in Singapore has the potential to be contaminated with *Vibrio* and *Salmonella*, thus proper handling at food service establishments should be required to ensure food safety. There is also a need to implement effective control measures to prevent cross contamination during post-harvesting seafood processing.

P3-76 Effect of Cryogenic Freezing on *Salmonella* and *Listeria* Recovery from Inoculated Shrimp

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Introduction: In the United States, shrimp is number one in seafood consumption. This product is usually imported frozen and the U.S. Food and Drug Administration has isolated *Salmonella* spp. from this frozen product.

Purpose: This research was conducted to determine the effect of two cryogenic freezing protocols (time and temperature) on the recovery of *Salmonella* and *Listeria monocytogenes* (LM) from inoculated shrimp and the color and texture after freezing.

Methods: Thawed shrimp were dipped in either a cocktail of *Salmonella* or LM to achieve a background level of 3 logs. The inoculated shrimp were frozen at -175°F (-115°C) for either 3 or 5 minutes. Recovery of the *Salmonella* was done on XLT-4 and LM on Palcam after 2, 9 and 21 days frozen storage at -20°C.

Results: The *Salmonella* or LM recovered after 2 days storage was 1 log lower than the initial inoculation on the shrimp and this recovery level remained constant for the 9 and 21 day stored sample. Texture analysis comparing the treated (frozen) and untreated shrimp showed no texture difference. The color analysis of the treated (frozen) and untreated showed no visual change.

Significance: The freezing protocols of -175°F (-115°C) for either 3 or 5 minutes had no effect on quality, whereas both cryogenic freezing times only slightly reduced the level of *Salmonella* or LM occurred.

P3-77 Distribution and Inactivation of a Human Norovirus Surrogate and Hepatitis A Virus in Oysters

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Introduction: Viruses account for more than 67% of foodborne illnesses. Currently, the FDA classifies seafood as one of the highest risk foods for virus contamination. In the U.S., approximately 7.6 to 14.5 million illnesses are attributed to the consumption of seafood each year. Oysters are associated with most of these cases. However, the distribution and inactivation of viruses in oysters is poorly understood.

Purpose: The objective of this study is to compare the distribution of a human norovirus surrogate (murine norovirus, MNV-1) and hepatitis A virus (HAV) in oyster tissues and to determine the effectiveness of heating to inactivate viruses.

Methods: Oysters were cultivated in salted water containing MNV-1 or HAV. At different time points, individual oyster tissues such as gills, stomach, and muscles were isolated, and the amount of virus present was quantified by plaque assay. The effectiveness of virus inactivation by heating was determined in both cell culture medium and oysters.

Results: We systematically determined the kinetics of distribution of MNV-1 and HAV in oyster tissues. Within the first 24 hours of cultivation, high amounts of viruses were detected in the gills, but not in the stomachs or muscles. The viruses in the stomachs and muscles gradually increased after 24–48 hours of incubation. At 72 hours, the virus titer in the gills and stomachs were significantly higher than in the muscles. In medium, both MNV-1 and HAV were resistant to heat treatment (50–70°C). In oyster tissue, viruses were more difficult to inactivate. Ongoing experiments are aimed to determine the optimal temperature and holding time to effectively inactivate the viruses without significantly affecting the quality of the oyster tissue.

Significance: Our results suggest that oysters act as filters and vehicles in accumulating foodborne viruses. An optimal thermal processing condition will effectively inactivate virus contaminants and reduce oyster-associated illnesses.

P3-78 Cooking Times and Temperatures for Safe Consumption of Louisiana Blue Crabs

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Introduction: Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Listeria monocytogenes and *Salmonella* species are the most common bacterial causes of foodborne outbreaks associated with Louisiana blue crabs. A steady increase in foodborne illness associated with crab consumption occurring at private residences is evidence that there is a lack of scientifically-derived, consumer-focused guidelines for safe preparation of Louisiana blue crabs.

Purpose: The purpose of this research was to determine the least amount of time and temperature needed to reduce or eliminate the aforementioned bacteria from Louisiana blue crab(s) with either boiling or steaming heat treatments.

Methods: Louisiana blue crabs were inoculated with *L. monocytogenes* (Lm F4260 CDC, Atlanta), *S.* Typhimurium (ATCC 14028), *V. cholerae* (ATCC 14035), *V. parahaemolyticus* (ATCC 33847), or *V. vulnificus* (ATCC 27562) and subjected to boiling or steaming time points with the internal temperatures recorded by ACR SmartButton® temperature loggers. After cooling, the crabs were picked, diluted with PBS, and plated in duplicate. The agar plates were incubated overnight at 37°C and examined after 24 hours for colony growth. The results were based on the amount of bacterial log reduction of each heat treatment time point.

Results: Time and temperature results were: boil one crab four minutes, cool one additional minute for a minimal internal temperature of 79.5°C ($P < 0.001$); steam one crab for five minutes, cool two additional minutes, for a minimal internal temperature of 57°C (P:0.0084); boil four crabs for 10 minutes, cool five additional minutes for a minimal internal temperature of 85°C ($P < 0.0001$); steam four crabs for 15 minutes, cool five additional minutes for a minimal internal temperature of at least 85°C ($P < 0.0001$).

Significance: The results of this study will be distilled into easy and concise cooking instructions for consumers to use as a guide for safely preparing Louisiana blue crabs.

P3-79 Use of High Hydrostatic Pressure Processing on Three Human Pathogenic Strains of *Vibrio parahaemolyticus* in Live Gulf Coast Oysters *(Cassostrea virginica)*

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Introduction: Few studies have been performed on the sensitivity of clinical *Vibrio parahaemolyticus* to high hydrostatic pressure processing (HPP) in *Cassostrea virginica* oysters. Ten clinical strains known to have caused foodborne illness outbreaks were obtained from the FDA and USDA. Phase One of the study determined the parameters that completely inactivated *Vibrio parahaemolyticus*, in the stationary phase, using various pressures and hold times. The second phase involved propagating the three most resistant strains, using the strains to contaminate Gulf coast shell oysters, and processing the contaminated oysters to determine the efficacy of HPP to inactivate the bacteria in oysters. The three most piezotolerant strains selected for assimilation in oysters and HPP treatments were DIE 12-052499, TX 2103, and ATCC 17802.

Purpose: The purpose of the study was to provide information on the effectiveness of HPP in reducing *Vibrio* illness.

Methods: Cell suspensions were grown to stationary phase (7 $\log_{10}$) in T1N1 broth, pelleted cells were reconstituted in PBS and 4 ml of each strain was sealed in transfer pipets. The pipet bulbs were subjected to HPP with all combinations of the following pressures and hold times 35,000, 40,000, 45,000, and 50,000 psi (241, 276, 310, and 345 MPa) and 5 hold times (1, 2, 3, 4, and 5 min). Twenty mls of a bacterial concentration of 10^8 CFU/ml were used to inoculate tanks containing oysters, resulting in a final concentration of $\pm 10^5$ cells/ml seawater. After 18 hours, the oysters were placed into vacuum bags and sealed. The oysters were then subjected to the HPP parameters listed above. After HPP, the oysters were shucked, and the FDA's BAM preferred laboratory procedures for microbiological analyses of seafoods was followed for *Vibrio* isolation and identification.

Results: For V. p. DIE 12-052499, HPP parameters of 345 MPa for 2 min and 310 MPa for 3 min both resulted in a 4.2 log reduction. For TX 2103, HPP parameters of 310 MPa for 2 min, and 345 MPa for 2 min resulted in a 4.2 log reduction. For ATCC 17802 from each pressure and time parameter, 17802 was inactivated using 276 MPa for 2 min, 310 MPa for 2 min and 345 MPa for 1 min all resulting in a 4.0 log reduction.

Significance: From the three most piezotolerant strains, a pressure of 310 MPa (40,000 psi) with a 3 min hold time at room temperature inactivated the pathogen. The level of oyster uptake of the *Vibrio* inoculum was 5.0 log, however the HPP parameters listed above could easily inactivate clinical *Vibrio* spp. in oysters at concentrations greater than 5.0 log. Sensory panels have evaluated oysters at both 310 and 345 MPa and the oysters were found to be acceptable.

P3-80 Effect of Antimicrobial Peptides on In Vitro and In Vivo Growth and Survival of *Vibrio* spp.

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Introduction: The antimicrobial peptide (AMP) Nisin is increasingly being employed by the food industry to inhibit and/or remove gram-positive pathogens from foods. However, effectiveness of this AMP against gram-negative organisms has traditionally been limited. Fortunately, recent research has shown that application of certain chemical or physical stressors leading to increased permeability of the gram-negative outer membrane allows for AMPs to become effective against these bacteria.

Purpose: These studies examined the effectiveness of the AMPs hepcidin and Nisin with and without chemical or physical stressors against *V. vulnificus, V. parahaemolyticus* and *V. cholerae* in in vitro assays and a live oyster model.

Methods: Growth inhibition of the bacteria by the two AMPs with and without EDTA or cold shock were evaluated in microtiter format and measured by changes in optical density of the AMP exposed cultures compared to controls. These same cultures were also evaluated for bacterial death by performing plate counts. A previously established oyster model was used to test the effectiveness of Nisin with and without cold shock on *V. vulnificus* in oysters.

Results: V. vulnificus was shown to be sensitive to hepcidin at various inocula (10^3–10^5 CFU/mL), but only at high hepcidin concentrations (50, 75 and 100µM). Nisin, when used alone, had no effect on growth or survival of the *Vibrio* spp. Additionally, when cultures were exposed to EDTA in conjunction with Nisin, there was no significant reduction in bacterial populations. However, concomitant cold shock and Nisin exposure resulted in a nearly 3-log reduction of the culture concentrations. The synergistic effect of cold shock and Nisin on bacterial concentration in oysters also showed some reduction of *V. vulnificus* numbers, but results were not as dramatic as in vitro assays.

Significance: Results from these studies offer promising potential for the application of a hurdle-type post-harvest processing treatment for oysters.

P3-81 Characterization of a Thermal Stable Antigenic Marker Protein for Fish Detection

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Introduction: A previously developed monoclonal antibody (MAb) 8F5 is specific to all cooked extracts of 55 common fish species without cross-reactivity with land animals, shellfish and food additives proteins tested. The antigenic protein of MAb 8F5 is a 36 kDa thermal-stable soluble protein, commonly distributed in fish muscle. Due to its thermal-stability, similar molecular weight, and wide distribution in animal muscle, the antigenic protein was speculated to be the myofibril protein, tropomyosin.

Purpose: The specific objective of this study is to further characterize this 36 kDa antigenic protein for MAb 8F5 along with fish tropomyosin.

Methods: The 36 kDa protein was extracted from cooked (100°C, 15 min) salmon, and tropomyosin was isolated from raw salmon according to published methods. Both 36 kDa protein and tropomyosin were purified by ion exchange chromatography. These protein samples were subsequently separated by gel electrophoresis in presence or absence of urea followed by blotting with MAb 8F5. Amino acid analysis and protein sequencing of a fragment were performed for both protein samples and compared with that of published data.

Results: The immunoblot results showed that MAb 8F5 recognized both 36 kDa protein and fish tropomyosin. The molecular weight of the 36 kDa protein and fish tropomyosin shifted from 36 kDa to 50 kDa in gel electrophoresis with urea. This molecular weight migration is one of the major characteristics of tropomyosin. The amino acid composition of 36 kDa protein is >99% identical with fish tropomyosin. A fragment of 12 amino acid residues of the 36 kDa protein is 100% identical to the counterpart of fish tropomyosin. Based on these results, the antigenic protein of fish-specific MAb 8F5 is confirmed to be tropomyosin.

Significance: The ubiquitous tropomyosin is likely to have the fish-specific region recognized by MAb 8F5 and can be used as a marker protein in an immunoassay for fish detection.

P3-82 Development of a Real-time PCR Assay for Detecting Histamine-producing *Clostridium perfringens* in Fish

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Introduction: Scombrotoxin (histamine) fish poisoning (SFP) is one of the most frequently reported illnesses associated with the consumption of seafood. Previous research has indicated that *Clostridium perfringens* may be a significant contributor to anaerobic histamine-formation in scombrotoxic fish. A real-time PCR method was developed as a rapid and specific tool to further investigate the role of this bacterium in SFP.

Purpose: The objective of this study was to develop a real-time PCR method for detecting histamine-producing (HP) *C. perfringens* in fish.

Methods: A primer set and a TaqMan style MGB fluorogenic probe were developed from a conserved region of the histidine decarboxylase gene of *C. perfringens*. The specificity of the real-time PCR assay was tested against 23 HP *C. perfringens*, 53 HP and 66 non-HP fish isolates. The efficiency of this assay was determined from standard curves of boiled *C. perfringens* template in the presence of 0.1% peptone, fluid thioglycollate medium and a 1:10 dilution (fish tissue: 1% NaCl) of mahi-mahi (*Coryphaena hippurus*) or Spanish mackerel (*Scomberomorus maculatus*).

Results: The real-time PCR assay detected all 23 HP *C. perfringens* isolates and none of the other 116 HP and non-HP isolates. In pure culture, the efficiency of the assay with/without internal amplification control was 102%. The efficiency of the assay in the presence of 0.1% peptone, fluid thioglycollate medium, Spanish mackerel and mahi-mahi matrix was 96%, 95%, 90% and 99%, respectively.

Significance: The real-time PCR assay developed in this study is a rapid and specific tool for detection of HP *C. perfringens*. This assay may serve as an important tool for investigating the role of *C. perfringens* in anaerobic histamine production in fish.

P3-83 An Exploratory Comparison of Knowledge Levels of Diverse Populations within a Food Safety Workshop

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Introduction: Food safety training conducted in workshop formats is a widely accepted practice within the industry. A basic assumption of these types of workshops is that students gain knowledge during the process. Researchers evaluated similar workshops with diverse populations in order to determine if knowledge acquisition varied based on the cultural origins of the participants. Conclusions determine methods and importance of improving future training programs.

Purpose: The purpose of this experiment was to determine the relationship of participants' knowledge and satisfaction of two pre-harvest food safety workshops with international attendees.

Methods: The data were collected from two food safety workshops. Workshop A attendees were comprised of feedlot managers. Workshop B contained members attending an international cattle conference. At the conclusion of each clinic, participants were administered a post-test analyzing knowledge gained during the presentations. In addition to the post-test, students answered a questionnaire designed to measure satisfaction.

Results: Results from the post-test were analyzed using an independent-samples t test. There were no significant differences between the two knowledge post-tests (t =.863, f = .03). However, from a practical standpoint, students gained more knowledge in Workshop A (M = 29.90, SD = 4.75) than in Workshop B (M = 24.12, SD = 4.26). There was a significant difference of workshop satisfaction between the two groups (t = .029 f = 5.02). Workshop participants were more satisfied with Workshop A (M = 91.47, SD = 16.24) than Workshop B (M = 69.83, SD = 22.21).

Significance: Workshop effectiveness continues to be a priority for all participants. Low performance in separate populations should be explored and corrected. Further research is needed to determine if differences in knowledge and satisfaction scores may be attributed to cultural differences between the international and U.S. participants as Workshop B had a large international population.

P3-84 Effectiveness of Educational Interventions to Improve Older Adults' Food Safety Practices

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Introduction: Older adults are at a higher risk for foodborne illness and are more likely than any other age group to experience complications, hospitalization, and death as a result of infection.

Purpose: The purpose of the study was to develop Web-based and print materials and evaluate the effectiveness of these materials at improving older adults' food safety practices.

Methods: The study employed a randomized, controlled design with participants assigned to an intervention group (n = 148) or control group (n = 124). Participants in each group completed Web-based surveys at pre- and postintervention.

Results: Study participants preferred the print materials and found them useful, informative, and a credible source of food safety information. The intervention group significantly reduced consumption of many foods associated with foodborne illness, including cold hot dogs ($P < 0.05$), cold deli meats ($P < 0.001$), raw or undercooked meat or poultry ($P < 0.001$), and deli salads ($P < 0.001$). The control group significantly reduced consumption of raw or undercooked meat or poultry ($P < 0.05$). The between-group difference in change was not significant for any of the foods asked in the survey. Statistically significant changes were observed among the intervention group for proper cooking of eggs. For example, the percentage of respondents who fried eggs until both the yolk and whites were firm increased by 21% for the intervention group (from 44% to 54%, $P < 0.05$) and increased by 4% for the control group (from 49% to 51%, n.s.); however, the between-group difference in change was not significant.

Significance: Targeted educational interventions are needed to educate older adults about food safety. Educators can use the study findings and conclusions to improve upon or develop and deliver effective foodborne illness prevention messages targeted to older adults.

P3-85 Assessing Food Safety Practices Contributing to Food Safety Culture

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Introduction: Based on risk assessments and better understanding of factors contributing to foodborne illness outbreaks, the retail food industry should consider shifting its focus to a behavior-based food safety system. By changing employee behavior to better handle all food safety issues, restaurants will ultimately be providing safer food and may reduce the risk of foodborne disease to consumers. In this respect, it is clear that, even though a restaurant may have the best food safety standards in place, if those standards are not practiced, they are not going to be effective (Yiannas, 2008).

Purpose: The objective of this study was to identify key factors that can influence food service workers to follow the food safety regulations and create a positive food safety culture.

Methods: A convenience sample of 103 (52 men and 51 women) foodservice employees was selected. Employees were given a survey containing 49 items and asked to evaluate statements about practices related to food safety training, managers' and employees' commitment to food safety, food safety practices within the establishment, and employees' food safety behavior on a Likert-type scale (1=strongly disagree; 5=strongly agree).

Results: Results indicated that employees took responsibility for food safety in their workplace, knew both when and why to wash their hands, changed gloves when needed and understood why they needed to change them. They believed that their job performance could affect the safety of the food the customer receives. Employees reported that management took food safety seriously showed leadership by keeping employees focused on food safety and visibly showed support for food safety by role modeling appropriate food safety behaviors.

Significance: The results showed that a strong commitment from management influenced the attitudes and behavior of employees. With proper training and education, employees practice proper food safety procedures.

P3-86 Factors Affecting Worker Food Safety Behavior: A Path Analysis Approach

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Introduction: Management commitment to food safety has been identified as being critical to the successful implementation and maintenance of a food safety management system. Recently, it has been shown that the relationship between management commitment to food safety and worker food safety behavior is fully mediated by other factors.

Purpose: The purpose of this study was to identify and discuss specific aspects of management's commitment to food safety and of mediating factors that predict worker food safety behavior.

Methods: Three focus group interviews and 27 in-depth interviews at nine meat establishments generated 405 pages of single-spaced verbatim transcripts which were analyzed using NVivo 8 software; field observations were conducted at 5 of the plants. The findings were used to develop a survey that was administered at four further-processing plants using single-stage cluster sampling. A path analysis was conducted on 472 surveys, which represented a response rate in excess of 91%.

Results: The path analysis identified the primary elements from the qualitative themes that have predictive capacity with respect to worker food safety behavior. Themes of leadership, food safety training, food safety program support factors, group norms, and supervision/monitoring of behavior were of particular relevance.

Significance: An understanding of the factors that are predictive of worker food safety behavior will enable interventions for improvement to be appropriately targeted.

P3-87 Evaluation of Current Food Safety Practices Used in Operating and Maintaining Hot/Cold Self Serve Bars in Retail Establishments

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Introduction: The increasing popularity and availability of foods offered by grocery stores in hot/cold self-serve bars has created new food safety training challenges.

Purpose: To determine the efficacy of current food safety training related to the operation of hot and cold bars foods in retail by: 1) evaluating current food safety practices in the operation of self-serve food bars, and 2) determining if food safety training topics need to be emphasized or added to current training materials.

Methods: Three retail chains participated by having 15 stores within each chain observed by the investigators on 2 days (pre- and post-training) at set-up, lunch time, and closing to collect information on employee food safety practices. After the pre-training observation, managers from 8 of the 15 stores within a chain attended an eight-hour food safety training course (treatment stores), while managers from the remaining seven stores received no additional training (control stores). Collectively, 90% of the managers that attended the training session passed the certification exam. Treatment store managers were encouraged to train their employees with the knowledge they had gained. Following the training session all stores were observed to collect post-training data.

Results: Objective one: The observational scores for the control and treatment stores were compared for both the pre-training and post-training visits. There was not a significant difference between the control and treatment stores' scores. This indicated that the current training practices did not add any significant positive change to the stores' performance. Objective two: The post-training scores were used to determine the food safety training needs. A minimum performance score of 75% indicated that the current food safety training practices were sufficient for that category. The categories for food temperature, utensil usage, and product handling, had post-training scores below 75% (60.24%, 74.24%, and 61.98%, correspondingly). These data indicate that training materials focused on these categories need to be developed.

Significance: Manager training did not significantly impact employee food safety practices. Improved food safety training methods and materials targeting employees need to be developed.

P3-88 Assessment of Food Safety Educational Needs and Current Practices of Front-line Grocery Food Handlers through Survey and Observational Data Collection

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Introduction: Grocery store associates in deli-bakery departments serve and prepare an increasing number of ready-to-eat foods and full meals. The shift in the retail food business increases the need for specific and effective food safety training programs in retail grocery establishments to prevent foodborne illness.

Purpose: This research examines food safety knowledge, training preferences, needs, and current practices of grocery store deli-bakery food handlers in Southwest and Southern Virginia.

Methods: This research had two phases. Phase I: employees completed a 34 question needs assessment survey eliciting information on demographics, food safety training needs, preferences and knowledge. Phase II: 15 employees' (from Phase I locations) food behaviors were observed for approximately six hours each. Observational data collection focused on cross-contamination, glove use and hand washing.

Results: Most grocery food handlers desired frequent hands-on, interactive one-on-one training that is short: less than two hours in length. The target audience's largest knowledge gaps included correct temperatures for cooking, reheating and cooling foods. While greater than 95% of participants reported correct hand washing techniques, observational behavior data showed less than 50% of hand washing events observed were correct. Additionally, food handlers were witnessed commonly not washing hands prior to putting gloves on, as well as practicing bare hand contact with ready-to eat foods.

Significance: Observed behaviors did not correlate with food safety knowledge. The creation of short, hands-on or interactive trainings for retail grocery food handlers that focus on changing food handling and preparation behaviors may enhance the food handler's safe food handling practices.

P3-89 Raw Meat and Poultry-specific Knowledge Gaps among Chicago Restaurant Meat and Poultry Food Handlers

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Introduction: Raw meat and poultry are potentially hazardous, commonly handled and cooked foods in restaurants. A knowledge survey of restaurant food handlers (N=509) was performed in Chicago restaurants (N=159) during 2009. The overall mean knowledge score was only 72%. However, when calculating each handler's knowledge score, inclusion of duty-unrelated food safety questions may bias results towards demonstrating lower food safety knowledge and may over represent the possible risk to the consumer.

Purpose: The purpose of this analysis was to compare the overall mean knowledge score to a task-specific knowledge score calculated only from questions relevant to meat and poultry food-handling duties.

Methods: Of the 509 restaurant food handlers interviewed, 373 meat and poultry food handlers were included in this analysis (73%). Of 44 knowledge questions, 13 questions were specific to raw meat or poultry handling and were used to create the task-specific knowledge score.

Results: The mean task-specific knowledge score was 72% (9.4/13) (the same as the overall knowledge score mean from the entire study). Of the 244 food handlers who had ever taken a food safety training course, 35.4% (95% CI = 29.3% - 41.3%) answered that meat can be stored anywhere in a refrigerator as long as it is wrapped in plastic, 36.2% (95% CI = 30.2% - 42.2%) did not know that eating ground meat that is not completely cooked can cause bloody diarrhea, and 19.9% (95% CI = 14.9% - 24.9%) thought that it is safe to put frozen chicken breast on the counter to thaw.

Significance: Despite limiting knowledge survey questions to the ones most relevant to the duties of restaurant meat and poultry food handlers, the knowledge score for restaurant food handlers was still no greater than 72%. These results underscore the need for educational interventions for restaurant food handlers including those with a history of certification.

P3-90 Lessons Learned Recruiting Restaurant Food Handlers to Participate in a Food Safety Survey: Implications for Future Research

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Introduction: The risk of acquiring foodborne diseases may arise from inadequate food handler knowledge and specific food handling behaviors. Developing appropriate study methods to approach the food handler population and adequately evaluate knowledge is essential.

Purpose: We determined appropriate methods to recruit and study food safety knowledge of restaurant food handlers.

Methods: We performed two survey studies. In the first, 729 food handlers from 211 restaurants in suburban Chicago, Illinois, were recruited by trained survey research employees during June 2009 through February 2010. To determine the best method to approach restaurants, initially 25 restaurants were recruited by contacting managers in-person and 25 by telephone. A 50-question survey was administered to food handlers in either English or Spanish and contained 40 food safety knowledge questions. In the second study, suburban restaurants were approached in person by either a public health sanitarian or a university research assistant during March through May 2010 and 94 food handlers were interviewed in 33 randomly selected restaurants. Food handlers were administered the same survey instrument.

Results: Restaurant participation rates differed substantially when approached in person (70%) than when approached by telephone (6.3%). As a result, the remaining restaurants were approached in person by researchers. At the conclusion of the study, the participation rate was 49%. Restaurant participation rates also differed substantially when approached by public health sanitarians (65%) than when approached by research assistants (34%). However, once restaurants agreed to participate, food handler participation was nearly 100% for both methods. Restaurants preferred to be approached during the hours just after lunchtime and preceding the dinner rush ("down time").

Significance: This study reveals best practices for recruiting restaurants for food handler food safety research. Approaching restaurants in person during "down time" was more successful. Collaboration between local health departments and food safety researchers may maximize restaurant participation in future studies.

P3-91 Evaluation of Hygienic Practices and Efficacy of an Educational Hand-washing Intervention among Restaurants in Lubbock and San Francisco

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Introduction: Proper hand washing technique is considered one of the most important practices to reduce pathogen transmission and prevent cross-contamination during food preparation, specially in food-service establishments.

Purpose: To determine the efficacy of a hand washing educational intervention program on the reduction of microbial indicators on hands and evaluate the effects of environmental sanitation associated with washed hands.

Methods: Hand swabs (n=370) from food services workers and environmental swabs (n=185) from preparation areas were collected in 37 restaurants (fast food and casual dining) in two separate cities of the United States before and after an educational intervention on proper hand washing techniques. Environmental swabs primarily included preparation tables, faucet handles, refrigerator handles, and doors. Swab samples were plated onto Petrifilm aerobic count plates and Petrifilm *E. coli*/Coliform count plates to determine numbers of microbial indicators. The training, approximately 30 minutes in length, included a hand washing video together with a hands-on hand washing practices, and explanation of proper use of gloves and hand sanitizers.

Results: APC recoveries were 3.65 CFU/hand and 3.72 CFU/hand ($P = 0.3597$), while coliform loads were 0.2 CFU/hand and 0.27 CFU/hand ($P = 0.3162$) in pre-training and post-training groups, respectively. Additionally, food workers in the San Francisco restaurants harbored significantly higher APC in comparison to those in Lubbock ($P < 0.0001$); furthermore, food workers in the casual dining restaurants had significantly higher APC (0.21 log CFU/hand) than those in the fast food restaurants ($P = 0.0067$). With regard to environmental swabs, faucet handles had the highest APC and coliform counts, with 8.69 and 4.66 log CFU/handle, respectively.

Significance: This study indicates that an educational intervention may not significantly reduce the total microbial indicators on food workers' hands in Lubbock and San Francisco. However, hands may become contaminated from environmental areas carrying high microbial loads rather than a result of improper hand washing.

P3-92 Development of a Food Safety Social Marketing Campaign

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Introduction: Children have a higher risk for foodborne illness compared to adults due to their underdeveloped immune system, lower body weight and lower control over meal preparation. Focus groups and a survey examining food handling beliefs, knowledge, and practices based on the Health Belief Model indicated families with young children were unsure/unaware of leftover food safety.

Purpose: To develop a food safety social marketing campaign based on results of focus groups and survey conducted with families with children.

Methods: Based on USDA-recommended storage time for leftovers, a slogan was developed: 4 Day Throw Away. A mascot, a large red number 4, was created and was the main character who conveyed the 4 Day Throw Away message. Social marketing drove the development of the campaign. Traditional media (press releases, radio PSAs) and #4 appearances at grocery stores were conducted. Magnets with the slogan and website were given to the targeted audience. Posters were developed and distributed with tear-off sheets that directed people to a website for additional information. The website (4daythrowaway.com) contains four videos with interactive polling, and food safety myths. In the videos, the mascot helps families understand the importance of using leftovers within 4 days or throwing the items away. Evaluation methods include website metrics and qualitative data from #4 appearances.

Results: Website visits from traditional media sources (news releases, TV and in-store appearances) were 436 for the first four weeks of the campaign and continue to increase. People are surprised at the four day message, ask specific questions about food storage and indicate throwing food items away.

Significance: To reach young audiences with important information on food safety topics, unique (non-traditional) methods are needed. This traditional social marketing campaign incorporated a website to disseminate a food safety message to the targeted audience.

P3-93 Watching the People Who Watch Your Kids: Observation of Childcare Workers' Hand Washing Frequency and Quality

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Introduction: There is a high rate of infectious illness in public child care centers, which are attended by approximately 3.74 million children in the United States per year. Infrequent and non-thorough hand washing behaviors among child care workers increases the risk of transmission of *Shigella sonnei*, norovirus and foodborne illnesses to children, which cause diarrhea and gastrointestinal illnesses.

Purpose: The purpose of this study was to observe current hand washing practices among childcare center workers in order to determine intervention points to improve hand hygiene and food safety.

Methods: Childcare workers' hand washing procedures were observed during normal working hours in N = 21 childcare centers in a large mid-Western city. Opportunities for hand washing were determined *a priori* based on national accreditation standards. Observers noted the frequency and thoroughness with which workers washed their hands in these opportunities. Fourteen opportunity types were observed including diaper-changing, before and after food preparation, after contact with bodily fluid and others.

Results: Hand washing opportunities (N = 1177) were observed in N = 47 rooms across N = 21 centers. Compliance was greatest for diaper changing (66.7%); pre-food preparation compliance was 29.5%. On average, workers washed their hands in 40% of the specified opportunities. When workers did wash their hands, they washed for an average of 9.56 seconds, and used soap 78.7% of the time, typically scrubbed the fronts and backs of their hands; after washing, workers dried their hands 34% of the time.

Significance: This study demonstrates that hand washing compliance among childcare workers is lacking, similar to that in the foodservice and health care industries. Persuasive communication interventions are needed to increase hand washing frequency and thoroughness in order to prevent the spread of foodborne illness to children, parents, and other childcare workers.

P3-94 Implementation and Assessment of Interactive Food Safety Educational Materials in Secondary Science

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Introduction: New food safety education materials were developed and introduced to secondary science educators through a workshop in the first phase of this two-phase study. The ready-to-implement interactive materials included a presentation on food microbiology, case studies on foodborne illness outbreak investigations, a video on laboratory principles for detection and identification of foodborne microorganisms, web-based games, and other supporting resources.

Purpose: For the second phase of this study, the food safety materials were evaluated for implementation ease in the classroom and impact on student knowledge.

Methods: Five teachers of high school microbiology/biology, emerging diseases, agriscience, advanced foods, or college microbiology agreed to participate in the implementation study. Teachers agreed to implement at least two components of the educational materials, administer a student test before and after use of the materials, and complete a questionnaire on ease of implementation. The 50-question student test format was multiple choice or true/false and covered topics in food microbiology, microbial detection and control, regulatory issues, and safe food handling practices. The teacher feedback questionnaire consisted of 20 questions to rate the materials on a 5-point scale on quality, ease of implementation with existing curriculum, and student enthusiasm.

Results: Of the materials provided, the introductory presentation on food microbiology and illness surveillance and the case studies on outbreak investigations were the most commonly implemented. Teachers rated the materials good to excellent on all measures of quality, implementation ease, and student interest. An increase in correct student responses to more than 60% of test questions was observed after exposure to the educational materials as compared to prior to use pre-test results.

Significance: Food safety educational materials centered on surveillance and investigation of foodborne illness outbreaks were readily implemented into the existing curriculum and positively impacted student familiarity and interest in microbiological food safety.

P3-95 Integration of Molecular Pathogen Detection Technology into an Undergraduate Food Science Curriculum—A Pilot Study

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Introduction: As the need for accurate, rapid pathogen detection methods increases, the food industry has begun utilizing molecular-based detection methods to verify safe products. There is a significant need to prepare students in food science curricula with the skills necessary to execute and evaluate these modern testing methods thus enhancing food safety.

Purpose: The goal of this study was to introduce Polymerase Chain Reaction (PCR) and PCR-based methods of foodborne pathogen detection into the undergraduate Food Science curricula at Purdue University.

Methods: This study was implemented into concurrent food microbiology laboratory and lecture courses for a two week period. Outcome-based learning objectives were defined prior to instruction. Pre- and post-instruction assessments were administered to quantify learning gains. Throughout this study, attendance was recorded and small group activities were implemented to encourage class participation. Students were asked to voluntarily complete a Student Assessment of Learning Gains (SALG) survey online aimed to assess their impression of their learning gains.

Results: A total of 54 (93.1%; n=57) students attended at least 80% of the lectures. Lecture evaluation scores significantly increased from an average of 30.5% at pre-assessment to 80.9% post-assessment (n=56 students; $P < 0.01$); 94.8% of students demonstrated improvement. Similarly, there was a significant difference in laboratory pre-assessment scores from an average of 19.8% to 54.3% (n=39 students; $P < 0.01$) where 82.1% of students improved. Based on SALG outcomes, 84% (44/52) of students expressed moderate to great enthusiasm for the topic, 75% (38/50) expressed interest in taking additional courses, and 83% (42/50) considered PCR and molecular-based detection technologies important to the food industry.

Significance: This study demonstrates successful integration of PCR-based detection methods in undergraduate food science curricula and indicates it may be a valuable tool to recruit and train undergraduate students for food safety careers in industry.

P3-96 Real Raw Milk Facts: An Innovative Evidence-based Food Safety Website

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Introduction: Despite public health warnings, the demand for raw (unpasteurized) milk appears to be increasing, especially among consumers interested in "health foods." In 2009, the International Association for Food Protection (IAFP) and the American Veterinary Medical Association (AVMA) each sponsored groundbreaking symposia addressing science, policy, and legal issues regarding raw milk consumption and sales.

Purpose: There is an urgent need for improved communication strategies to the public, media, and lawmakers regarding the risks and benefits of raw milk consumption. The Internet is used increasingly by the public to research health-related topics. We noted that an Internet search on "raw milk" produced conflicting and confusing information weighted heavily toward raw milk advocacy groups. To address this imbalance, we created an innovative evidence-based website, www.realrawmilkfacts.com, that specifically targets consumers likely to seek raw dairy products. The website serves as a clearinghouse for factual raw milk-related informational materials.

Methods: The website was built using the WordPress publishing platform; the Google Analytics tool was used to track statistics on daily number of visitors to the site. The content was developed through input from diverse professionals including food safety scientists, public health officials, legal experts, and social scientists. Data were compiled regarding outbreaks and illnesses resulting from milk consumption and scientific studies describing the risks and benefits of raw milk consumption. A set of frequently asked questions was developed with feedback from raw milk consumers. Video testimonials of families severely affected by raw milk-related illness were posted on the website to "put a face" on the risks of raw milk consumption.

Results: The website was launched on April 15, 2010, simultaneous with a major raw milk article in USA Today. The number of visits peaked at 1,811 on the launch date. A total of 37,439 visits have been logged through January 18, 2011, with an average of 138 per day. Real Raw Milk Facts Dot Com is consistently on the first or second page in a "raw milk" Google search.

Significance: Outbreaks and illnesses linked to consumption of contaminated raw dairy products are an ongoing public health problem. Our website presents important factual information on raw milk risks and benefits in an easily accessible format designed to inform consumers most likely to seek out raw dairy products. The website may serve as a model in using the internet for other food safety education efforts. The evidence-based food safety educational website developed in a public-private collaboration following IAFP and AVMA raw milk symposia, has been successful. In the future, we will further expand our outreach by using social networking sites such as Twitter and Facebook in conjunction with the website.

P3-97 Development of Comprehensive Risk Reduction Protocols to Enhance the Microbiological Quality and Safety of Artisan Cheeses

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Introduction: Recently, the U.S. Food and Drug Administration has increased inspections across the entire U.S. cheese industry, an industry increasingly comprised of specialty, artisan and farmstead cheese operations. Smaller operations are often considered higher risk due to a lack of resources, capital and technical expertise to implement effective control programs.

Purpose: The Vermont Institute for Artisan Cheese provided one-on-one technical assistance to small-scale Vermont artisan cheese makers to aid in the development of individual risk reduction protocols.

Methods: Detailed documentation of the facility and traffic patterns was used to develop environmental monitoring plans. Sponge samples were collected from target areas and analyzed for presence of *Listeria* species. Results were used to develop corrective actions and procedures to prevent pathogen reentry and cross-contamination. Comprehensive review of the complete cheese making process was conducted and samples of raw milk, curds, whey and finished cheese were collected for physicochemical and microbiological analysis. All samples were screened for *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7 and *Staphylococcus aureus*. Follow-up visits were conducted to assess the implementation of recommendations and to collect samples for follow up analysis.

Results: Seventeen of the 34 (50%) eligible businesses participated. *S. aureus* was the most common pathogen isolated from samples. No *Listeria* spp. or *Salmonella* spp. were detected in any of the milk samples tested. *E. coli* O157:H7 was isolated from one milk sample as well as the final product manufactured from that milk. A total of 326 environmental sites were initially tested with 28 and 3 positive for *Listeria* spp. and *L. monocytogenes*, respectively.

Significance: Data from follow-up visits verified that implementation of recommendations and corrective actions aided in the elimination and control of the *Listeria* spp. Survey responses indicated that the program increased producer awareness of quality and safety issues and helped businesses to better understand and manage these risks.

P3-98 Consumer Response to New Methods of Washing Produce

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Introduction: Since foodborne illness has been linked to fresh produce, some consumers are interested in what they can do at home to increase food safety. One risk reduction strategy is to adequately wash produce. Washing with ozonated or electrolyzed water has reduced bacteria levels for some items in laboratory settings.

Purpose: This study explores consumer awareness of food safety issues related to produce, assesses interest in learning about produce washing techniques, and measures response to washing with ozonated or electrolyzed water produced by commercial equipment.

Methods: Consumer attitudes were explored through focus groups (n=10) of 100 consumers conducted in Northern California in 2010. Attitudes were analyzed by content analysis using Xsight while response to key questions were quantified using a Likert scale.

Results: Concern about produce safety was moderate, 3.2 (1=Very Unconcerned and 5=Very Concerned), with some consumers highly concerned and others viewing foodborne outbreaks related to produce as rare. Participants were given a brief written description of each water treatment which included how the process worked and information on safety. Most participants were comfortable with the new technologies and considered them safe, but would not purchase the units for themselves. Likelihood to purchase the ozonated water generator was 3.5 and the electrolyzed water generator was 4.5 (1=very likely, 5=very unlikely). Participants believed that water preparation would add an unnecessary step to food preparation and the units were too expensive, large and cumbersome. Many would consider this approach in the future if the systems were more user-friendly and fast-acting. Participants did support use of an advanced washing system in foodservice.

Significance: This study suggests that consumers are unlikely to embrace an approach that requires them to deviate from their traditional food handling practices. Preparing a special solution and soaking produce even for a few minutes was considered too time consuming.

P3-99 Effectiveness of Food Safety Training Video for Volunteers in Faith-based Organizations

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Introduction: During large disasters, faith-based organizations (FBOs) have provided food and shelter for evacuees. Despite potential food safety risks due to non-professional food handlers preparing and serving food, only limited training has been provided for these operations.

Purpose: The purpose of this research was to implement and evaluate effectiveness of a 20-minute food safety training video developed for FBO members.

Methods: Participants were 183 FBO members from 11 faith-based organizations in KS, AL, FL, and NC. An identical pre- and post-test instrument was developed, reviewed by experts for content validity, and pilot-tested prior to intervention and assessment. The questionnaire included food safety knowledge, attitudes toward food safety risks and training, and demographic questions. Attitude questions used 7-point scales, and knowledge questions used multiple-choice and multiple answer formats. Prior to data analyses, responses to knowledge questions were re-coded to 1 for correct and 0 for incorrect answers (Max 30).

Results: After watching the training video, the average attitude score increased significantly (4.96 ± 0.38 vs. 5.25 ± 0.46, $P < 0.001$). More specifically, participants' perceived barriers toward food safety training were reduced after watching video (3.46 ± 1.10 vs. 3.08 ± 1.15). Prior to training, participants lacked food safety knowledge related to proper storage (3.05 of 6.0), handwashing (9.55 of 13.0), food acquisition (1.93 of 3.0), proper cooking (1.70 of 3.0), and personal hygiene (2.14 of 5.0). After watching the video, food safety knowledge scores improved significantly (all $P < 0.001$). The total knowledge score improved from 18.38 ± 4.35 to 24.39 ± 3.62 ($t = -24.62$, $P < 0.001$).

Significance: Results were not significantly different based on gender, age, and education levels indicating the program is effective across different demographic groups. FBO leaders and food safety educators may use this program to train non-professional volunteers and FBO members to improve their food safety knowledge for evacuation shelter operations and other congregational functions.

P3-100 Consumer Use and Understanding of Preparation Instructions for Prepared, But Not Ready-to-Eat Meat and Poultry Products

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Introduction: Consumers are increasingly relying on prepared, but not ready-to-eat (NRTE) meat and poultry products because they are convenient, quick, and easy. Recent foodborne illness outbreaks suggest that some consumers are not properly preparing these foods to ensure food safety.

Purpose: To understand consumers' use and understanding of preparation instructions for frozen, NRTE meat and poultry products.

Methods: We conducted 11 focus groups with consumers in five U.S. cities. The groups were segmented by at-risk population (e.g., parents of children less than 5 years old and adults aged 60 and older).

Results: Some participants do not distinguish among different products and/or brands and have the misperception that all frozen meat and poultry products are ready-to-eat (RTE) and do not require cooking for safety. Most participants always read preparation instructions when preparing a product for the first time, and many participants believe that it is important to follow these instructions to ensure the quality of the finished product. Regarding labeling statements such as "pull back film and stir" and "let stand in the microwave," many participants do not understand that the purpose of the instructions is to ensure food safety and do not always follow the instructions word for word. Most participants do not follow instructions regarding making adjustments to microwave cooking time because they do not know the wattage of their microwave or believe the stated cooking time is sufficient. Most participants do not use a food thermometer to check the doneness of frozen food products, but instead rely on past experience and their senses to determine doneness.

Significance: The focus group findings suggest the need for consumer education or labeling changes to motivate consumers to properly follow preparation instructions to ensure food safety, and thus help reduce foodborne illness from consumption of prepared, but NRTE meat and poultry products.

P3-101 Effects of Storage Practices on Microbiological Quality of Irrigation Water from Two Community Gardens

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Introduction: Community gardens provide educational opportunities and alternative food sources for the neighboring families. Educating community farmers of Good Agricultural Practices will enhance safety of their products.

Purpose: The purpose of this study was to assess the effects of storage conditions on the microbiological quality of creek water used to irrigate the crops by two community gardens.

Methods: Samples of irrigation water from two independent community gardens were collected from the field storage tanks. The same water samples were also stored in laboratory and greenhouse environments. Coliform and *E. coli* counts, pH, temperature and carbon dioxide of the water samples were continually monitored for two weeks. The experiments were repeated for four subsequent cycles.

Results: The temperatures of water stored in the greenhouse were on average 7.2°C higher than water in the field storage tanks and 9.4°C higher than water stored in the laboratory. In general, the pH of water increased 1.1 and carbon dioxide concentration decreased 1.2 mg/L over the experiment periods. The increase of water pH was significantly less (0.6) in the laboratory compared to other locations. The average initial coliform count was 890 CFU/100 mL and the average minimum days required to reach a safety threshold of 100 CFU/100 ml were 7 days in the field tanks and in the laboratory and 4 days in the greenhouse. The decrease of *E. coli* count followed similar trends.

Significance: Higher temperature and direct sunlight had significant impacts on the survival of coliform bacteria. Storage time was crucial in reducing bacteria contamination in irrigation water. A recommendation was made to these community farmers to store water at least a week in the field tanks before use for irrigation.

P3-102 Consumer Attitudes toward Food Safety in Asian and Mexican Restaurants

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Introduction: Dining out is a frequent activity for most Americans. Ethnic restaurants like Chinese and Mexican are the most sought after dining experience after American cuisine. The Centers for Disease Control and Prevention (CDC) foodborne outbreak data from 1990–2000 shows an increase of foodborne outbreaks from ethnic restaurants.

Purpose: This study was designed to explore consumers' perception of restaurant food safety and attitudes toward ethnic restaurants. This information can be used to guide ethnic restaurant owners in being more aware of their customers' needs.

Methods: Focus groups and an online survey were conducted to explore consumer perceptions of restaurant food safety and attitudes toward ethnic restaurants. Questions were designed to explore consumer awareness of food safety issues, concerns for dining in ethnic food establishments, opinions on current food safety practices in restaurants, and suggestions on how to improve food safety practices.

Results: Twenty-seven Californians participated in focus groups, and volunteers in California and Florida, N=994, completed an internet survey. While respondents noted that they only dine at restaurants where they trust the food, Mexican restaurants scored higher in food safety confidence than Asian restaurants. Consumers believe that kitchen cleanliness is the most important factor influencing safety followed by restroom cleanliness and cooking temperature. People believe all restaurant employees should be trained in food safety, restaurant inspections should be frequent and unannounced, and the government should be more active in protecting consumers.

Significance: Food safety is an underlying factor in restaurant selection and continued patronage. Alerting restaurant employees to consumer attitudes through food safety training courses and elsewhere could help ethnic restaurant respond to consumer expectations and build customer loyalty.

P3-103 Attitudes of Consumers Regarding Safety Certification in Restaurants in the Municipality of Campinas, Brazil

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Introduction: Quality certifications aim both to ensure the safety of food and also inform consumers about those attributes. For effective communication with the consumer, it is necessary to analyze their attitude towards safety certificates.

Purpose: The objective of this study was to analyze the attitudes of consumers towards the private safety certifications in restaurants.

Methods: The collection of data was conducted in the city of Campinas, State of São Paulo, Brazil, through interviews with 350 adults who eat out of their homes and who have already heard about quality certifications. The scale used was previously validated and is composed of 24 sentences, distributed in 5 factors (the certification and safety of the food, substitutes for certification, the importance of certification, certification and information to consumers). The consumers indicated their level of agreement with the statements through a 7-point scale. Socioeconomic information was also collected. Basic statistical descriptions and average comparative tests (Student's *t*-test and ANOVA) were performed using SPSS version 17.0 software.

Results: The mean attitude was 5.3 ± 0.7, indicating agreement with the certification. In the comparison between factors, the factors 3 and 4 showed averages of 5.6 ± 1.2 and 6.2 ± 0.9, respectively, indicating a larger contribution of those factors to the positive attitude. When the averages were compared, no significant differences were observed between genders ($P = 0.353$), educational background in the area of health ($P = 0.240$), level of education ($P = 0.105$) or family income ($P = 0.539$). Among the age ranges, a significant difference was observed ($P = 0.04$). The average attitude of subjects younger than 24 years of age was significantly lower than the average of individuals between the ages of 40 and 50 years.

Significance: These results showed that consumers have a positive attitude regarding certification, thus reinforcing the need to stimulate both the development and the promotion of certifications aimed at food safety.

P3-104 In-home Observation of Consumer Use of Food Thermometers to Measure Endpoint Temperature of Ground Beef Patties

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Introduction: Proper use of a food thermometer to determine doneness in ground beef patties is considered the only reliable method to ensure patties have reached a safe temperature. However, only 15% of consumers use a food thermometer to judge doneness of hamburgers. Motivating and educational materials are needed to increase the number of consumers who use a food thermometer correctly to safely cook ground beef patties.

Purpose: The methods used by consumers to cook and form ground beef patties at home and their use of two types of food thermometers to measure cooked patty temperature were observed to facilitate a redesign of food thermometer education and motivation materials.

Methods: Consumers were recruited via flyers placed in public areas. Volunteers (25) participated in a video-taped in-home interview in which they formed two patties from bulk ground beef and cooked the patties sequentially according to their normal practice. Patty temperature was measured by the participants with a food thermometer (dial-type with one patty, digital with the other) when they judged cooking to be complete. A researcher verified patty temperature with a thermocouple. Participants were also asked about thermometer use and answered a short questionnaire.

Results: Fourteen men and 11 women formed patties averaging 111 g (± 32 g) in size (range 56 to 194 g) from a 500 g package of bulk ground beef and cooked the patties for an average of 8 min 50 s (± 2 min 15 s) (range 3 min 23 s to 13 min 28 s). When measuring patty temperature, few consumers inserted the thermometer through the side (dial = 5, digital = 4), which provides the most accurate reading; most inserted it through the top, either straight down (dial = 8, digital = 9) or at an angle (dial and digital = 12). Dial thermometer readings averaged 19 °C (± 16 °C) lower than the thermocouple measurement of patty temperature, indicating the 5 cm sensing area had not been fully inserted. For digital thermometers (1.3 cm sensing area), the difference was only 4 °C (± 5 °C). According to the thermocouple measurement of patty temperature, 58% did not reach the safe endpoint of 71.1 °C. Prior to the interviews, three-quarters of the participants had not considered using a thermometer to check doneness of hamburgers.

Significance: Educational materials to encourage consumer use of thermometers with ground beef patties should emphasize the requirement to insert the entire sensing area of a dial thermometer stem into the patty. Since both dial and digital thermometers are widely available to consumers, manufacturers should clearly indicate the temperature sensing area of the stem and provide clear instructions for use.

P3-105 Addressing Food Technologists Shortages in South Wales Using Bursary Placement Scheme

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Introduction: The Global food industry is facing a sharp decline in the number of food scientists and technologists whose innovative work on product development is vital. The Sector Skills Council for Food and Drink found that 20% of food and drink manufacturers in Wales currently employ Food Scientists and Technologists (FSTs) (estimated at 114 FSTs). Two thirds of Welsh FST employers consider there to be a shortage in supply to these occupations and there has been an increase in demand in the last 3 years which is expected to continue in the future.

Purpose: One of the Food Industry Skills Project (FISP) objectives is to address the shortages of Food Scientists and Technologists in Wales.

Methods: The scheme was piloted in 2 companies in South Wales, both of which had a supported graduate each for 3 months during the summer period. Both placements were in the quality department and both undergraduates had a clear set of objectives to work to and achieve. The steering group came up with the idea of a bursary placement scheme to help fill some of these gaps in the short term. The scheme is designed to: 1. Up-skill and prepare food technology undergraduates for the sector and a food environment facilitating the transition from studying Food Science and Technology to applying learning to the workplace. 2. Support food industry with quality or technical issues. The scheme provides a structured support system of knowledge and expertise for the company including coordinated prearranged meetings to monitor and guarantee the desired end result.

Results: The scheme resulted in the development of a new product in Company A and an improved food safety management system in Company B. Both companies have signed up to the scheme for 2011. Both students demonstrated clear learning outcomes from the placement and are looking forward to another summer placement before graduating.

Significance: This is an improvement to the way placements used to work, plus it allows us to assist companies with technical and quality issues more effectively. The scheme is set to achieve a minimum of 6 placements during the summer of 2011 and subsequently 12 placements in 2012.

P3-106 Characterization of a Thermally Stable Amylase for Use as a Time-temperature Indicator (TTI)

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Introduction: Enzyme-based Time Temperature Indicators (TTIs) have proven to be a feasible and reliable tool for validation and optimization of pasteurization processes. Several enzymes have been characterized on basis of heat inactivation kinetics and shown to have Z-values in same range as those of bacterial spores.

Purpose: The main objective of this study is to characterize the kinetic properties of a thermally resistant amylase produced by the hyperthermophilic organism *Pyrococcus furiosis* and compare the activity with widely recognized test organisms.

Methods: Dried amylase preparations isolated from *P. furiosis* were provided by Unilever Inc. Enzyme was resuspended in a buffer solution with 0.4 mM EDTA at pH 6.0 for testing. Activity of this treated enzyme was determined using a commercial starch (Phadebas) at 80°C. Vm and Kmax were determined by varying substrate concentrations. Thermal resistance was determined by treatment at three different temperatures 119°C, 121°C, 125°C for 3, 6, 9, and 12 min.

Results: Measured rates of reaction resulted in a Vmax= 7.6745 and Km= 6.8775. D values of enzyme were as follows: D119 = 18.76 min, D121 = 7.95 min, and at D125 = 5.02 min. The published D value for *Clostridium botulinum* spores at 121.1°C is reported to be 3 min.

Significance: Understanding the kinetic properties of this thermally stable enzyme along with its D-values will allow its use as a TTI in place of currently used TTIs such as *Geobacillus stearothermophillus*. Use of an enzyme-based TTI instead of a microorganism can significantly reduce the time and expense of validation and optimization of thermal processes.

P3-107 Develop a Practical HACCP Implementation Model for SMEs through Knowledge Transfer (Extension) in Line with BRC Global Standard for Food Safety

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Introduction: One of the most serious issues facing the industry is the sharp decline in the number of food scientists and technologists whose innovative work on food safety and HACCP implementation is vital. The Sector Skills Council for Food and Drink found that 20% of food and drink manufacturers in Wales currently employ Food Scientists and Technologists (FSTs) (estimated at 114 FSTs). Two thirds of Welsh FST employers consider there to be a shortage in supply to these occupations and there has been an increase in demand in the last 3 years which is expected to continue in the future.

Purpose: To address this issue, University of Wales Institute Cardiff has been given the opportunity to offer flexible knowledge transfer partnerships or 'extensions' to assist SMEs in achieving a number of technical improvements to the business by building tri-partnerships with a food graduate, a group of experts and a food business utilizing this relationship to apply knowledge and expertise.

Methods: Over the past 2 years the knowledge transfer experts have developed a method to effectively implement HACCP studies and plans in line with the British Retail Consortium (BRC) Global Standard for Food Safety (issue 5). This applied model is a concise, structured, systematic, comprehensive manual within which a company (with the support of a partnership) can implement the Codex Alimentarius HACCP Principles, cross referenced with the BRC Standard. The model allows for individual company interpretation and flexibility with a thorough, fully implemented and maintained structure to their food safety management system.

Results: The model and its key elements were successfully implemented into more than 10 companies across food sectors such as meat, bakery, fruit juice, ice cream, ready meals and fermented meats. The program has resulted in a reduction in the number of food graduates leaving the geographical area with a consequential impact on food business operations.

Significance: The financial impact and knowledge exchange outputs delivered by this program will be presented in the form of case studies at the presentation.

P3-108 Food Regulations: What a Beginning 105 Years Ago? An Educational Primer Import and Export Mandates

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Introduction: Food safety regulations and legislation have been put in place to protect consumers and guide producers. However, an extensive amount of information exists throughout varied resources and can be time consuming to find, along with adding to confusion for consumers about which agency is responsible for which mandate, and how food safety regulatory agencies interact. To add to this dimension of confusion, minimal understanding exists about inter-workings of international and domestic food safety regulations.

Purpose: The purpose of this study is to clarify and educate persons about historical (circa 1906) and recent food regulations. This educational informational study will provide an organized synopsis for practitioners and consumers to better understand existing regulations in the food safety industry. A primary focus is on importation and exportation regulations.

Methods: An in-depth synthesis of literature on domestic and international mandates was conducted from credible sources: governmental agency websites, business law and food safety law references. Educational methodologies are used to organize and convey information for consumers and practitioners.

Results: Regulation exists on multiple levels. A marking point in food safety legislation of the United States (U.S.) can be seen as early as 1906 with the Pure Food and Drug Act, and the 1906 Meat Inspection Act. The Federal Food, Drug and Cosmetic Act (1938) in relation to adulterated food states: "shipment, distribution and sale of adulterated food, and false mislabeling of food, is prohibited." Several Amendments were made to this act, the Food Additives Amendment (1958), Color Additives Amendment (1960) and Animal Drug Amendment (1968). Legislation and amendments have provided consumer protection to ensure no adulterated food is allowed for sale and distribution, and proper labeling. U.S. and international countries have varying legislation requirements/allowances. Importing food into the U.S. can be an extensive process, while exporting foods can be conducted with an "export exemption." The U.S. federal agencies that have a major role in regulation of imported foods are discussed. These agencies' food jurisdiction responsibilities and interactions in cross-national situations are also discussed.

Significance: This study is timely and based on the theme of the 100 year Anniversary of the International Association for Food Protection. This study provides a convenient and relevant primer for informing consumers and practitioners of vital historical strides and policy inter-workings that have proved valuable to our improving food safety worldwide.

P3-109 Fate of *Salmonella* spp. and *Listeria monocytogenes* in Nine Different Types of Minimally Processed Vegetables

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Introduction: A significant increasing trend has been observed in the last two decades linking the occurrence of foodborne disease and fresh produce (FP). *Salmonella* and *L. monocytogenes* (*L.m.*) are two of the pathogens challenging the microbiological safety of FP.

Purpose: The purpose of this study was to determine the fate of *Salmonella* and *L.m.* in different types of minimally processed vegetables (MPV) stored at different temperature conditions.

Methods: Nine different MPV (escarole, collard greens, cabbage, spinach, watercress, arugula, carrot, lettuce and yakisoba) testing negative for both pathogens were acquired from retail in São Paulo, Brazil. Triplicate packs (25 g) of each MPV were inoculated at 10^3 CFU/g with a cocktail of five strains of *Salmonella* spp. (two *S.* Typhimurium, *S. enterica* O:47:z4,z23:-, *S.* Infantis and *S.* Concord) and *L.m.* (1/2b and 4b) and packed using modified atmosphere (5% CO_2, 15% O_2 and 80% N_2). Packs were stored at I= 100% of the shelf life (6 days)/7°C; II= 30% at 7°C and 70% at 15°C; III=100% at 15°C. *Salmonella* and *L.m.* were enumerated in the beginning and at the end of storage using MLCB and Oxford agars, respectively (LOD: 10^1 CFU/g). Two different trials were performed and results expressed the difference (γ) between initial (No) and final (Nf) populations.

Results: Both pathogens grew on all MPV, except cabbage and carrots. An increase in $\gamma \geq^1$ log CFU/g was observed only at conditions II and III. The best substrates for the growth of *L.m.* were collard greens ($\gamma \geq^3$ log CFU/g) and arugula ($\gamma \geq^2$ log CFU/g) at conditions II and III. Escarole and arugula represented the best substrates for growth of *Salmonella* ($\gamma \geq^2$ log CFU/g), at all storage conditions.

Significance: The knowledge of the fate of *Salmonella* and *L.m.* on different types of MPV under different storage scenarios is relevant to gather information on their behavior and will be useful to improve MPV safety.

P3-110 Fate of *Salmonella* and *Listeria monocytogenes* on Fresh-cut Celery

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Introduction: Fresh-cut celery is considered to be ready to eat without any further consumer preparation. *Salmonella* and *Listeria monocytogenes* have been reported on fresh-cut celery. Little is known about the impact of consumer handling practices on the fate of these pathogens in this product.

Purpose: Our objective was to determine the fate of *Salmonella* and *L. monocytogenes* on fresh-cut celery under different storage conditions.

Methods: A cocktail of rifampicin-resistant *Salmonella* or nalidixic acid-resistant *L. monocytogenes* was spot inoculated onto either the cut or uncut celery surface at a level of ca. 3 log CFU/g. Samples were stored in either sealed zipper-top plastic bags or sealed polyethylene containers at 4, 12, or 22°C for up to 7 days. Samples were homogenized and pathogens enumerated on non-selective and selective agar supplemented with an antibiotic.

Results: Salmonella and *L. monocytogenes* populations decreased over 7 days at 4°C, under all conditions, by ca. 1 log CFU/g (0.1-0.2 log CFU/g/day reductions) and 1–2 log CFU/g (0.1–0.3 log CFU/g/day reductions), respectively. *Salmonella* and *L .monocytogenes* populations remained stable at ca. 3.0 to 3.5 log CFU/g for 7 days at 12°C under all conditions. All *Salmonella* populations increased by ca. 1.5–2 log CFU/g over 48 h at 22°C, under all conditions, with the greatest growth (2.1 log CFU/g) observed on the cut surface of samples stored in polyethylene containers. *L. monocytogenes* populations remained stable ca. 3 log CFU/g for 48 h at 22°C, under all conditions.

Significance: When held under refrigerated temperatures, *Salmonella* and *L. monocytogenes* do not grow on fresh-cut celery. *Salmonella*, but not *L. monocytogenes*, has the potential to grow on temperature-abused fresh-cut celery, especially if inoculated onto the cut surface.

P3-111 Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on Fresh-cut Watermelon Stored at 4 and 23°C

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Introduction: Fresh-cut watermelon is considered ready to eat and does not require additional preparation by the consumer. However typical consumer handling practices may have an effect on pathogen growth and survival.

Purpose: The objectives of this study were to quantify the impacts of storage times, temperature, storage container type and covering of cut watermelon on the fate of *Salmonella* and *E. coli* O157:H7.

Methods: Whole watermelons were cut into flesh cubes or triangular wedges with the rind intact. Twenty gram samples were weighed into either zipper-top plastic bags or polyethylene containers. A five-strain cocktail of rifampicin-resistant *Salmonella* or *E. coli* O157:H7 was spot inoculated at 10^3 CFU/ml onto exocarp (green rind), thick exocarp (white rind) or mesocarp (pink flesh). Containers were left open, closed with a lid, or covered with plastic wrap and stored at 4 or 23°C. Pathogens were enumerated at intervals up to 7 days, after homogenizing the watermelon and plating onto tryptic soy agar supplemented with rifampicin. DMFit software was used to quantify pathogen growth rate.

Results: E. coli O157:H7 and *Salmonella* grew rapidly on all wet, cut watermelon surfaces at 23°C, with most growth rates falling in the range of 0.20–0.30 log CFU/h regardless of packaging. Neither pathogen grew at 4°C, regardless of inoculation site or storage conditions. When inoculated on the rind and stored in open containers, bacterial populations declined. However, when stored in covered containers *Salmonella* and *E. coli* O157:H7 grew at 0.45 and 0.29 log CFU/h, respectively and both reached a final population level of 10^6 CFU/g.

Significance: Maintaining fresh-cut watermelon at refrigeration temperature, regardless of storage conditions, prevents growth of foodborne pathogens on watermelon flesh. Storage of cut watermelon wedges in open containers prevents pathogen growth on the rind but not the flesh at ambient temperatures.

P3-112 Behavior of *Escherichia coli* O157:H7 in Packaged Spinach after Exposure to Sodium Hypochlorite

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Introduction: Leafy greens have been associated with multiple outbreaks of *Escherichia coli* O157:H7 (EC) infection in recent years. More information is needed on how EC is able to survive during post-harvest processes, including sanitizing washes with sodium hypochlorite.

Purpose: To evaluate EC survival during storage of packaged spinach after exposure of cells to sodium hypochlorite.

Methods: A green fluorescent protein-expressing strain of EC was exposed to a sub-lethal concentration of sodium hypochlorite (1 ppm) for 30 s at ambient temperature. Control cells were treated in phosphate buffer. Spinach (100 g) was inoculated with chlorine-treated or control cells at 4–5 $\log_{10}$ CFU/ml and then added to packages prepared from micro-perforated and non-perforated films to obtain high-oxygen (20%O_2 / 3%CO_2) and low-oxygen (0%O_2 / 15%CO_2) atmospheres, respectively. The packages were stored at 4°C or 15°C for 14 days. EC populations in triplicate packages were quantified by plate counts of fluorescent colonies at 4 - 5 sampling points during the 14-day storage. The data were analyzed by DMFit and Microfit to demonstrate the growth curve and kinetics.

Results: At 4°C, EC populations showed reduction rates of 0.055 and 0.020 log CFU/ml per day for the high- and low-oxygen packages, respectively, during the 14-day storage. At 15°C, EC populations increased in the low-oxygen packages, with growth rates of 0.106 log CFU/ml per day, whereas the high-oxygen packages showed 0.049 log CFU/ml reductions per day. There were no apparent differences in behavior between chlorine-treated and control cells at either temperature or package atmosphere, indicating that sublethally-stressed cells can grow as well as untreated cells during storage. Future studies will focus on the behavior of EC after inoculation on the spinach and exposure to chlorinated process water.

Significance: These studies will be useful for understanding pathogen survival and improving the effectiveness of post-harvest processes for leafy greens.

P3-113 Survival of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 on Inoculated Peanut and Pecan Kernels Stored at -20°C, 4°C and 23°C

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Introduction: Salmonella is currently being used as the target organism for nut and nut products due to previous outbreaks; however, little is known about the fate of pathogens on nuts other than *Salmonella* on almonds or pecans.

Purpose: The purpose of this study was to determine the effects of pathogen concentration and storage temperature on the survival of *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 on shelled peanuts and pecans.

Methods: Raw peanuts and pecan halves and pieces were inoculated separately with cocktails of nalidixic acid-resistant strains of *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7. Kernels inoculated at ca. 3, and 6 log CFU/g after a 24-h drying period were stored for 28 days at -20, 4 and 23°C. Kernels inoculated at ca. 5 log CFU/g after a 72-h drying period were stored for 181 days at -20, 4 and 23°C. Ten-gram subsamples from each condition were enumerated for the appropriate pathogen over the course of the storage by stomaching and plating on specific and non-specific media supplemented with nalidixic acid.

Results: Populations of all three bacteria on peanut and pecan kernels decreased 1 to 2 logs during the initial 72-h drying. Reductions of all bacterial populations during 28 days of storage at -20, 4 and 23°C on peanuts and pecans were not affected by inoculum level. When stored at -20°C and 4°C for 181 days, populations of *Salmonella, L. monocytogenes*, and *E. coli* O157:H7 remained stable at ca. 5 log CFU/g. At 23°C, linear rates of decline were 0.32, 0.55, and 0.40 log CFU/month on peanuts and 0.21, 0.83, and 0.45 log CFU/month on pecans for *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7, respectively.

Significance: Salmonella survives better than *L. monocytogenes* and *E. coli* O157:H7 on pecans and peanuts stored at ambient temperature, supporting its use as the target pathogen for nuts and nut products.

P3-114 Long-term Survival of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Almonds and In-shell Pistachios at Three Storage Temperatures

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Introduction: With the exception of data on the survival of *Salmonella* on almonds and pecans, very little is known about the behavior of foodborne pathogens on nuts.

Purpose: The purpose of this study was to assess the survival of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* on almonds and pistachios at common storage temperatures.

Methods: Almond kernels and in-shell pistachios were inoculated with 4- to 6-strain cocktails of nalidixic acid-resistant *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes* at 6 log CFU/g and dried for 72 h. Post drying, inoculated nuts (n = 6) were stored at -20°C, 4°C, or 23°C. Every 4 to 8 weeks for over 52 weeks, levels of each pathogen were enumerated by plating onto appropriate selective and non-selective agars containing 50 µg/ml of nalidixic acid. When levels fell below the limit of detection (0.3 log CFU/g), 10-g samples were enriched and then confirmed for the presence of the pathogen. Moisture content and a_w of uninoculated nut samples were also monitored throughout storage.

Results: As the inoculum dried, levels of all pathogens declined by 1 to 2 log CFU/g on both almonds and pistachios. During storage after drying, no decline in population levels was observed at -20 or 4°C for any pathogen. Rates of decline at 23°C were 0.22, 0.60 and 0.71 log CFU/month (almonds) or 0.25, 0.35 and 0.86 log CFU/month (pistachios) for *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes*, respectively. By 7 months of storage, levels of *E. coli* O157:H7 and *L. monocytogenes* approached the limit of detection but samples continued to be positive on enrichment. Moisture (4.5%) and a_w (0.45) were consistent during storage at -20 and 23°C but increased slowly at 4°C to 6% moisture and 0.60 a_w.

Significance: The persistence of *Salmonella* on almonds and pistachios reinforces the selection of this organism as the target pathogen of concern for nuts and their products.

P3-115 Behavior of Inoculated *Salmonella* spp. in Postharvest Pistachio Handling

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Introduction: Pistachios are harvested by shaking onto catch frames, hulled under wet, abrasive conditions, separated in float tanks and dried with hot air before further drying at ambient temperature in storage silos. *Salmonella* is a concern in tree nuts, but routes of contamination during and after harvest remain largely unknown.

Purpose: The objective of this study was to determine the behavior of *Salmonella* at different steps in the post-harvest handling of pistachios.

Methods: Fresh pistachios (in-hull or hulled; 50% moisture, 0.99 a_w) were collected from a California processor. Pistachios were inoculated with a six-serovar cocktail of nalidixic acid-resistant *Salmonella enterica* at 4 log CFU/g and stored at 23 or 35°C and 50 or 90% relative humidity (RH). Hulled in-shell pistachios were also inoculated at 4 or 8 log CFU/g, dried in a laboratory oven at 70°C to a target 15% moisture, and held at 23°C for 5 days. *Salmonella* populations were recovered by agitating pistachios in 0.1% peptone and plating onto tryptic soy (TSA) and bismuth sulfite agars (BSA).

Results: Within 6 h at 35°C, levels of *Salmonella* on in-hull pistachios increased by 2.1 or 1.6 log CFU/g at 90 or 50% RH, respectively. When stored at 23°C, increases of 1.9 or 0.83 log CFU/g were observed within 12 h at 90 or 50% RH, respectively. For hulled pistachios stored at 23°C, populations of *Salmonella* increased by 1 and 2 log CFU/g after 8 and 24 h, respectively, at both 50 and 90% RH. *Salmonella* populations decreased by 4 log CFU/g in pistachios dried to 15% moisture (0.86 a_w); thereafter, no significant change in levels of this pathogen were observed over 5 days of storage at 23°C.

Significance: These results indicate that time between harvesting, hulling, and drying should be minimized to reduce the opportunity for multiplication of *Salmonella* in post-harvest pistachios; however, the drying step is an opportunity to achieve significant reductions of this organism.

P3-116 Inactivation of *Salmonella* on Pecan Nutmeats by Hot Air and Oil Roast Treatments

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Introduction: Pecans are exposed to potential sources of foodborne pathogens in pre- and post-harvest environments. After separation from inedible nut components, nutmeats are dried with hot air and may also be dry- or oil-roasted, thereby enhancing microbiological safety.

Purpose: We undertook a study to determine the effectiveness of hot air and oil roast treatments in killing *Salmonella* on pecan nutmeats.

Methods: Pecan nutmeats (halves and medium pieces) were separately inoculated by immersing in a five-serotype suspension of *Salmonella* or by surface application of powdered chalk containing the pathogen. Inoculated stored nutmeats were adjusted to low (2.8 – 4.1%) and high (10.2 – 11.5%) moisture content and exposed to forced air at 60 – 170°C for up to 20 min; inoculated dry nutmeats were immersed in peanut oil at 110 – 138°C for up to 4 min. The number of *Salmonella* surviving treatments was determined.

Results: Air treatment at 120°C for 20 min reduced *Salmonella* in low- and high-moisture nutmeats by 1.18 – 1.26 and 1.89 – 2.04 log CFU/g, respectively. Reductions were >5 logs when dry nutmeats were treated at 150°C for 15 min. *Salmonella* was slightly more heat resistant in immersion-inoculated nutmeats than on surface-inoculated nutmeats. Exposure of immersion-inoculated pecan pieces to oil at 127°C for 1.5 min or 132°C for 1.0 min reduced *Salmonella* by 5 log CFU/g. Treatment of pecan halves at 132°C for up to 4.0 min did not always result in a 5-log reduction.

Significance: Hot air treatment of pecan nutmeats cannot be relied upon to achieve a 5-log CFU/g reduction of *Salmonella* without changing sensory qualities. Lethality of treatments is influenced by size of the nutmeat, moisture content, and way nutmeats become contaminated, i.e., immersion vs. surface inoculation. Treatment temperatures and times typically used to oil roast nutmeats are sufficient to reduce *Salmonella* by 5 log CFU/g.

P3-117 Evaluation of Critical Process Parameters for *Salmonella* Inactivation on Almonds Subjected to Thermal Pasteurization

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Introduction: Dry food pasteurization is significantly affected by product water activity (aw) and process conditions, but very few inactivation models account for these factors or have been validated for commercial-type processes.

Purpose: The objective of this study was to evaluate a novel inactivation model and multiple process validation methods for accuracy and uncertainty, via pilot-scale thermal processing of inoculated almonds.

Methods: Almonds were inoculated with *Salmonella* Enteritidis PT30 or *Enterococcus faecium* (NRRL B-2354) at ~10^8 CFU/g and equilibrated over salt solutions to a_w 0.25 or 0.85. The inoculated almonds were heated in a pilot-scale moist-air impingement oven (121°C; v_{air} = 2.7 m/s) with either dry or humid air (dew point < 33°C or = 69°C) to a targeted lethality of 4 log. Thereafter, surviving *Enterococcus* and *Salmonella* were enumerated (3 reps per treatment) by stomaching, diluting, and plating on deMan, Rogosa and Sharpe agar or modified trypticase soy agar (35°C, 48 h), respectively. Almond surface temperatures were measured (9 reps per treatment) via surface thermocouples (T_{surf}) and aluminum almonds (T_{Al}), as a physical surrogate, with these temperatures then used to calculate *Salmonella* inactivation using a traditional (D, z) model and a previously published modified model accounting for process humidity.

Results: The repeatabilities (standard errors of replication) of the computed and biological (*Enterococcus* and *Salmonella*) validations (in log CFU/g) were: traditional model (0.08) < modified model (0.13) < *Enterococcus* (0.33) < *Salmonella* (0.50). For dry air at a_w 0.25, the traditional model using both T_{surf} and T_{Al} over-predicted lethality ($P < 0.05$) by 1.67 and 1.34 log CFU/g, respectively, while the computed lethalities based on the modified model, using both Tsurf and TAl, and the *Enterococcus* results, were equivalent ($P > 0.05$) to the actual *Salmonella* lethality. At a_w 0.85, both the traditional and modified models under-predicted lethality compared to *Salmonella* ($P < 0.05$); however, the lethalities predicted by the modified model (using T_{surf} and T_{Al}) were closer (1.93 and 0.31 log CFU/g) than the traditional model (5.52 and 4.04 log CFU/g) to the actual *Salmonella* lethality results.

Significance: These results indicate that product a_w, process humidity, and the predictive model used all impact the accuracy and repeatability of thermal process validations for *Salmonella* inactivation on the surface of dry products.

P3-118 Evaluation of the Efficacy of Various Antimicrobial Treatments for Pistachios

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Introduction: Although foodborne outbreaks associated with pistachios are rare, several cases of illness related to the consumption of pistachios have been reported recently.

Purpose: This study evaluates the effectiveness of various antimicrobial interventions applied singly and in combination with heat to inactivate *Escherichia coli* O157:H7 and *Salmonella* when applied under laboratory conditions intended to represent application of the intervention at a key location in pistachio processing, namely as a spray after initial washing of freshly hulled pistachios and just prior to drying.

Methods: In-shell pistachios were inoculated with approximately 10^8 CFU/g of *E. coli* O157:H7 or *Salmonella* and treated by spraying for 30 seconds with various antimicrobials: acetic acid (2%), citric acid (2%), lactic acid (2%), peroxyacetic acid (80 ppm), hydrogen bromide (100 ppm), and water (as a control). Samples treated with chemicals were kept at room temperature for 30 minutes and were either analyzed for the effectiveness of microbial inactivation or subjected to an additional thermal treatment (130°F, 145°F, or 160°F) to reduce the moisture content of in-shell pistachios to 12% of the final mass. Following treatment, samples of pistachios were neutralized in buffer. Pathogen survival was determined by spread plating on selective media.

Results: Antimicrobial treatments of pistachios applied singly were not as effective in reducing levels of *E. coli* O157:H7 and *Salmonella* as were the combined treatments of the antimicrobial followed by heat (drying). Lactic acid (2%) was the most effective antimicrobial resulting in reductions in levels of *E. coli* O157:H7 and *Salmonella* of 0.25 and 0.33 log CFU/g, respectively. Antimicrobial treatments of pistachios using lactic acid, citric acid and acetic acid following by subsequent drying at 145°F and 160°F significantly ($P < 0.05$) resulted in significant reductions in pathogens of 1.16 to 1.57 log CFU/g for *Salmonella* and 1.51 to 1.63 for *E. coli* O157:H7.

Significance: This study can serve as a reference to further evaluate the effectiveness of antimicrobial treatments applied singly or in combination with thermal treatment in controlling the pathogen burden of pistachios.

P3-119 Antimicrobial Activities of a New Fresh Produce Sanitizer on *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* and Log Reduction on Indigenous Microorganisms under Commercial Conditions

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Introduction: With the recent number of recalls on fresh produce there is a need for a more effective sanitizer in lowering the risk of foodborne illness associated with vegetative pathogens like *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*.

Purpose: This presentation reviews the microbial efficacy of a new sanitizer that is composed of lactic acid and peracetic acid (LA-PA) on the three common vegetative pathogens: *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*. Log reduction on indigenous microflora in both the wash water and leafy produce generated under commercial setting would also be presented.

Methods: The sanitizer antimicrobial activity in solution and attached on leaves was demonstrated by suspended cells challenge test method and spot inoculation attached cells challenge test method, respectively. Water samples and leafy product samples were collected from LA-PA wash system to demonstrate the efficacy of the sanitizer in commercial application.

Results: The log reduction on the suspended 5-strain cocktail of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* was >6 logs, >6 logs, and >4 logs, better than chlorinated water, respectively. LA-PA also enhanced the reduction of three pathogens attached on spinach and romaine leaves by 1 log and 2 logs, respectively, when compared with chlorinated water. The log reduction of total aerobic plate counts by LA-PA during the processing of 10 different products under commercial condition was at least 2 to 3.5 logs better than that of chlorinated water.

Significance: LA-PA demonstrates significant improvement in reducing vegetative pathogens and indigenous microorganisms when compared with chlorinated water. Results indicate that LA-PA can lower the risk of foodborne illness in the fresh produce industry under both laboratory and commercial environments.

P3-120 Impact of Organic Load on Sanitizer Efficacy against *Escherichia coli* O157:H7 in Simulated Leafy Green Processing Water

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Introduction: In order to reduce the persistence and spread of bacterial pathogens in recirculating flume water during leafy green processing, chemical sanitizers must be continually added to counteract the negative impact of increasing organic load in the water.

Purpose: This study assessed 1) the ability of three commercial sanitizers to reduce *Escherichia coli* O157:H7 populations in simulated wash water containing various organic loads in a bench top system, and 2) the relationship between various physicochemical parameters and organic load in the wash water on sanitizer efficacy.

Methods: A 4-L glass carboy with a spigot was used to assess the efficacy of three commercial sanitizers (three concentrations each) - XY-12 (30, 50, 100 ppm free chlorine), Tsunami 100 (10, 50, 80 ppm peroxyacetic acid), and Tsunami 200 (10, 50, 80 ppm mixed peracid) (Ecolab, St. Paul, MN) in triplicate against a 4-strain avirulent, GFP-labeled *E. coli* O157:H7 cocktail in wash water containing 0, 1, 5 or 10% (w/v) blended lettuce solids, with sanitizer-free water serving as the control. After inoculating the water at 6 log CFU/ml, 50-ml water samples were collected through the opened spigot at 10-s intervals over 90 s and neutralized. Water was assessed for temperature, pH, Chemical Oxygen Demand (COD), Oxidation/Reduction Potential (ORP), total solids, turbidity, and maximum filterable volume (MFV) using a 0.45 µm membrane. The neutralized samples were appropriately diluted and surface-plated on TSAYE + amp with or without membrane filtration to quantify *E. coli* O157:H7.

Results: Using commonly employed concentrations of 50 ppm for both peroxyacetic and mixed peracid and 100 ppm for free chlorine, all three sanitizers significantly ($P < 0.05$) reduced *E. coli* O157:H7 populations by 5.88 log at a 0% organic load after 90 s of exposure. At a 10% organic load, the peroxyacetic- and mixed peracid-based sanitizers significantly ($P < 0.05$) decreased the pathogen by 3.88, 5.42, whereas reductions of 5.88, 0.25 and 0.07 log were seen for the chlorine-based sanitizer at organic loads of 1, 5 and 10%, respectively. Increasing organic load correlated with COD, total solids, MFV and turbidity ($P < 0.05$).

Significance: Overall, Tsunami 200 was least affected by organic load, followed by Tsunami 100 and XY-12 with COD, total solids, MFV and turbidity likely useful in assessing efficacy of the chlorine-based sanitizer.

P3-121 Use of Edible Plant Extracts as an Alternative for Decontamination of *Shigella* and *Escherichia coli* O157:H7 on Leafy Green Vegetables

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Introduction: Fresh cilantro, parsley and spinach have been identified as vehicles of transmission of enteropathogenic bacteria. Several outbreaks associated with consumption of contaminated leafy greens have been documented. Since these products are regularly consumed fresh, and current decontamination methods are not always effective, alternative strategies for their decontamination are required.

Purpose: The objective of this study was to determine the efficacy of plant extracts as alternatives for the disinfection of cilantro, parsley and spinach that have been artificially contaminated with *E. coli* O157:H7 and *Shigella sonnei*.

Methods: The extracts from several edible plants were obtained using ethanol as the extraction solvent. A micro-method was used to determine the minimal bactericidal concentration (MBC). Plant extracts exhibiting the lowest MBC were selected for further studies. Leaves of fresh greens were washed with sterile water and dried. For seeding, leaves were submerged in suspensions of bacteria, dried and stored at 4°C until use. To determine the effects of the extracts, leafy greens were submerged in a container and subjected to treatments with: chlorine, Citrol® or selected plant extracts. Each treatment systems was stored at 4°C (for 0, 1, 5 and 7 days), and bacterial counts were determined by plate count.

Results: From the 41 plant extracts tested, the extracts from oregano leaves and from the peel and pulp of limes were selected for detailed analysis. These extracts were found to be as effective as chlorine or Citrol® in reducing the population of pathogenic bacteria on leafy-greens by 1 or 2 logs.

Significance: These extracts could potentially be a natural and edible alternative to chemicals to reduce the risk of *E. coli* O157:H7 and *S. sonnei* contamination on leafy vegetables.

P3-122 Antibacterial Activity of Vinegar and Canola Oil on Experimentally Inoculated *Salmonella enterica* and Gram Negative Microflora of Bagged Spinach Leaves

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Introduction: Various strategies have been developed to reduce the bacterial burden on leafy greens. Consumers may be interested in using common household ingredients to further reduce numbers of bacteria on leafy greens used in salads.

Purpose: In this study we explored the use of vinegar, or vinegar and canola oil as a salad dressing, to reduce bacterial levels on bagged spinach leaves.

Methods: Fresh bagged spinach was purchased and stored at refrigeration temperature. Samples (25 g) of spinach leaves were weighed and placed in plastic bags. In some experiments, these were evaluated for existing microflora, and in other experiments they were inoculated by dipping in a solution containing a cocktail of *Salmonella enterica* or *Listeria monocytogenes* strains. Bags were incubated at room temperature with vinegar or vinegar and oil (10–30 ml final volume) for 10 to 40 minutes. Samples were removed, disrupted using a stomacher and the lysates diluted and plated on appropriate agar.

Results: Incubation with various types of vinegar, substantially reduced (> 3 $\log_{10}$ CFU) the predominantly gram negative microflora on packaged spinach leaves. A similar response was observed when white vinegar was mixed in various proportions with canola oil, as used in salad dressing. Mixing spinach leaves with vinegar or vinegar and oil for 20 minutes at room temperature resulted in a 1 to 2 $\log_{10}$ reduction in CFU of *Salmonella enterica* on experimentally inoculated spinach leaves. A modest reduction (approximately 0.5 $\log_{10}$ CFU) was observed when spinach leaves were experimentally inoculated with a cocktail of *L. monocytogenes* strains.

Significance: These findings suggest that mixing spinach leaves with vinegar and oil as a salad dressing can reduce the bacterial burden, including *Salmonella*, if it is present, on spinach leaves.

P3-123 Effect of Surface Topography of Alfalfa, Broccoli and Radish Seeds on Reduction of Inoculated *Escherichia coli* O157:H7 with a New Sanitizer-surfactant Combination

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Introduction: Surface roughness has been reported as one of the factors affecting microbial attachment and removal. Seed surfaces are complex and different seed varieties have different surface topographies. As a result, one sanitizer may be able to eliminate pathogenic bacteria on one seed but not on other seeds.

Purpose: The objectives of this research were to investigate the effectiveness of selected sanitizer-surfactant combinations on *E. coli* O157:H7 inactivation on alfalfa, broccoli, and radish seeds, to observe surface topography of the seeds, and to determine the correlation between them.

Methods: Surface topography of each seed type was measured by confocal laser scanning microscopy (CLSM) and surface profilometry. The obtained data were calculated into surface roughness (R^a) and used to quantitatively describe the seeds' surface topography. Seeds inoculated with *E. coli* O157:H7 were washed for 20 min in a sanitizer-surfactant solution at various concentrations and rinsed for 10 min in tap water.

Results: Radish seeds had the highest R^a values as measured by both methods, followed consecutively by broccoli and alfalfa seeds. Overall, a negative correlation existed between the seeds' R^a values and microbial removal at lower concentrations, while log reduction upon washing in high concentration and 20,000 ppm chlorine solutions did not show correlation with R^a. Moreover, there was an interaction between sanitizer and surfactant on *E. coli* O157:H7 inactivation on all three seed types, indicating a synergy between them. A combination of 10% sanitizer and 1% surfactant resulted in complete elimination of *E. coli* O157:H7 on alfalfa seeds, but not on other seeds.

Significance: The results indicated that different seed types had different surface topographical characteristics, contributing to discrepancies in the ability of sanitizers to eliminate *E. coli* O157:H7 on the seeds. Therefore, the effectiveness of one sanitizer on one seed type should not be translated to all seed varieties.

P3-124 The Use of Chlorine Dioxide Gas to Control *Alternaria alternata* and *Stemphylium vesicarium* on Roma Tomatoes

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Introduction: Early blight, caused by *Alternaria alternata*, and black spots on tomatoes, induced by *Stemphylium vesicarium*, are major plant diseases causing economic losses in growing fields, greenhouses and packing facilities. It has been challenging to control tomato deterioration during storage and more research is needed to develop better microbial control systems. Chlorine dioxide gas (ClO_2) is an innovative and powerful microbial inactivation technology.

Purpose: Determine ClO_2 gas treatment conditions for inactivation of *A. alternata* and *S. vesicarium* in vitro and vivo. Evaluate the potential application of the technology to control post-harvest spoilage on Roma tomatoes.

Methods: Antifungal activity of ClO_2 concentrations (10 mg/l) at different exposure times (1, 3, 5, 7 and 10 minutes) on mycelia growth in vitro conditions were evaluated in PDA Petri dishes. After treatments, plates were re-incubated at 28 °C for 1 week and radial growth calculated. The same treatments were also applied to study ClO_2 effects on conidial germination. Spores were enumerated after treatments on PDA media. These conditions were also used to estimate fungal disease development on treated and un-treated Roma tomatoes experimentally inoculated and stored at 28 °C for 7 days. Each set of experiments were performed in triplicate.

Results: Time was a significant factor for fungi disease control ($P < 0.05$). After 3 minutes of ClO_2 treatment, mycelia growth was completely inhibited for *A. alternata* and *S. vesicarium*. Similar results were observed on conidial germination. After 1 minute treatment, ~ 2.5 log spores/ml reduction was recorded and after 3 minutes the count was less than detectable values (0.7 log spores/ml). The efficacy of ClO_2 treatments was also studied in vivo conditions. While untreated Roma tomatoes developed white molds and black spots after 3 days of storage, produce decay was significantly delayed after 3 and 5 minutes of treatments for both diseases.

Significance: Results suggests potential application of ClO_2 to reduce postharvest diseases on tomatoes caused by *A. alternata* and *S. vesicarium*.

P3-125 Effect of Combined Treatments with Peroxyacetic Acid and High Power Ultrasound on *Escherichia coli* O157:H7 Cross-contamination

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Introduction: E. coli O157:H7 has been responsible for several outbreaks involving fresh leafy green vegetables. Peroxyacetic acid (POAA) has the potential to be an effective sanitizer in preventing cross-contamination during commercial fresh-cut produce washing. The addition of high power ultrasound (HPU) to the washing system may enhance the POAA efficacy.

Purpose: The purpose of this study was to reduce *E. coli* O157:H7 cross-contamination during commercial fresh-cut lettuce washing using a combined washing treatment of POAA and HPU.

Methods: Fresh-cut romaine lettuce leaves (25 cm^2) were inoculated with 50 µl 10^8 CFU/ml *E. coli* O157:H7 and air-dried in a biosafety cabinet for 60 min. Two leaves were washed in 2 L of 0, 30, 50, or 80 ppm POAA with or without HPU (400 W) for 2 min to measure the efficacy of the combined treatment. In addition, ten inoculated leaves were rinsed in 500 ml DI water for 2 min to produce contaminated wash water. To test for cross-contamination, the wash water was used to rinse ten uninoculated leaves for 2 min, and these leaves were then washed with 30 ppm POAA with or without HPU for 2 min. Recovered cells were plated on TSAYE agar and analyzed for reduction from leaves as well as cross-contamination to uninoculated leaves.

Results: Inoculated cells were washed from leaves by approximately 2.3, 3.8, and 4.4-log when treated for 2 min with 30, 50 and 80 ppm POAA and HPU, respectively. HPU consistently improved washing efficacy by up to 1.5-log. A high level of cross-contamination (4.5–log CFU/g *E. coli*) occurred after uninoculated leaves were rinsed in contaminated wash water for 2 min. A subsequent 2 min wash in POAA alone or in combination with HPU reduced counts by approximately 1.2-log and 2.3-log, respectively.

Significance: These results highlight the implication of cross-contamination during fresh produce washing, and indicate that the combination of POAA and HPU can reduce contaminating *E. coli* O157:H7 cells from the surface of fresh-cut lettuce leaves.

P3-126 Postharvest Intervention Methods and Combined Treatments to Decontaminate Spinach

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Introduction: A recent multi-state outbreak of *Escherichia coli* O157:H7 in baby spinach has increased consumer awareness of potential health risks associated with the consumption of fresh leafy greens.

Purpose: To ensure the safety of fresh baby spinach, it is essential to evaluate and optimize individual and combined efficacies of currently available decontamination methods.

Methods: Baby spinach leaves were inoculated with a five-strain cocktail of *E. coli* O157:H7 using spot or dip-inoculation methods, in order to compare the decontamination efficacy for both surface and infiltrated *E. coli* O157:H7 contamination, respectively. The sole and combined efficacies of ultraviolet (UV) light (254 nm, 12.5 – 1040 mJ/cm^2), mild heat wash (40°C - 50°C) and acidified sodium hypochlorite (pH 6; 10 - 200 ppm) treatments on reducing the population of *E. coli* O157:H7 were evaluated.

Results: UV light (500 ± 20 mJ/$^{cm2)}$ was shown to significantly reduce *E. coli* O157:H7 populations ($P < 0.05$) by 1.5 ± 0.05 log CFU/g in dip-inoculated samples. The population was reduced by 1.8 ± 0.03 log ($P < 0.05$) at 120 ±10 mJ/cm^2 for spot-inoculated spinach. Additional UV exposure did not yield any further reduction in *E. coli* O157:H7 populations ($P < 0.05$). Acidified sodium hypochlorite (pH 6) resulted in a 0.4 ± 0.03 log CFU/g reduction at a 10 ppm concentration. While there were no significant differences with an increased concentration of acidified sodium hypochlorite for the dip-inoculated samples, the 200 ppm treatment resulted in a 0.7 ± 0.05 log CFU reduction from spot-inoculated samples. The mild heat treatments negatively affected the organoleptic properties of baby spinach and did not significantly reduce the populations of *E. coli* O157:H7 when compared to control washed samples (20°C). Therefore, the mild heat treatment was not included in the combined decontamination process. The combined treatment of optimized UV (120 ±10 mJ/cm^2) and acidified sodium hypochlorite treatment (pH 6; 10 ppm) resulted in a total of 2.7 ± 0.05 log CFU reduction for both spot and dip-inoculated baby spinach.

Significance: These results indicate that the optimized combination treatment of UV and acidified sodium hypochlorite was significantly effective in reducing *E. coli* O157:H7 populations on baby spinach.

P3-127 Effect of Minimal Processing Steps on Microbial Population Profiles on Lettuce in a Processing Plant

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Introduction: Minimal processing may contribute to contamination of leafy green vegetables.

Purpose: The objective of this study was to determine contribution of each processing step on the microbial load of lettuce and the role of cross-contamination between product and food-contact surfaces. In addition, microbial population profiles on leaf surfaces throughout processing were investigated.

Methods: Lettuce samples and swabs of food-contact surfaces were collected at seven processing points in a commercial plant at multiple times during the processing shift. Samples were quantitatively assessed for total aerobic, coliform and generic *E. coli* counts. 16S rDNA-T-RFLP profiles were obtained for each sample.

Results: Total aerobic and coliform counts on lettuce differed at various stages of processing ($P < 0.001$): Washing initially decreased coliform and aerobic bacteria on products ($P = 0.001$ and $P = 0.092$, respectively), but both these indicators of microbial contamination in packed (finished) product were similar to pre-washed levels. *E. coli* was not detected. Counts on food-contact surfaces were the lowest before the processing began ($P < 0.001$). Peak contamination on food-contact surfaces was detected after two hours of processing, and then contamination declined following 4 h and 6 h of processing, without sanitation interventions. Given these data, the increased bacterial counts on the product were not likely a result of cross-contamination from surfaces. 16S rRNA-T-RFLP profiles remained similar on lettuce leafs throughout minimal processing indicating that minimal processing steps do not modify the microflora of unprocessed lettuce.

Significance: Elucidation of factors contributing to the increase in microbial populations in final processing steps will facilitate the development of strategies to enhance food quality and safety of minimally processed leafy green vegetables.

P3-128 Use of High Hydrostatic Pressure to Eliminate *Salmonella* on Jalapeño and Serrano Peppers

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Introduction: The 2008 multi-state salmonellosis outbreak caused by contaminated Jalapeño and Serrano peppers have raised serious concerns about the microbial safety of fresh or minimally processed hot chiles.

Purpose: The objective of this study was to evaluate the potential of using high hydrostatic pressure (HHP) technology for the decontamination of Jalapeño and Serrano peppers superficially or internally contaminated with *Salmonella*.

Methods: Jalapeño and Serrano peppers were sliced into quarters and halves, respectively. Slices were spot-inoculated or soak-inoculated with a diluted suspension of a five-strain culture cocktail of *Salmonella* to a final level of ~ 6 log CFU/g. Pepper samples were then subjected to pressures ranging from 300–500 MPa for 2 min at 20°C in a dry, pre-wet (dipped in water for 30 s) or pre-soaked (immersed in water for 30 min) state.

Results: Pressure inactivation of spot-inoculated and soak-inoculated *Salmonella* on dry, pre-wet and pre-soaked peppers increased as a function of the pressure magnitude with ca. 3.5–6.1 log CFU/g (Jalapeño) and 3.9–6.6 log CFU/g (Serrano) reduction in the population of *Salmonella* at 500 MPa, compared to 1.1–2.2 log CFU/g (Jalapeño) and 1.0–3.3 log CFU/g (Serrano) inactivation at 300 MPa. In addition, the inactivation efficacy of HHP also depended on the degree of wetness of samples and increased in the order of pre-soaked > pre-wet > dry state. When dry peppers were pressure-treated at levels of up to 500 MPa, a surviving population of *Salmonella* (2.6–2.7 log CFU/g) was detected. However, when the pepper slices were pre-wet or pre-soaked prior to HHP treatment under the same condition, the population was significantly reduced ($P < 0.05$) to below detectable limits.

Significance: This study highlights the potential of pressure-treatment of pre-packaged moistened Jalapeño and Serrano peppers to ensure complete inactivation of the pathogen to ensure a safe product on the retail market.

P3-129 Enhanced Resistance of Sanitizer-injured *Escherichia coli* O157:H7 on Baby Spinach during X-ray Irradiation

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Introduction: Commercial washing of leafy greens in sanitizer-treated flume systems remains standard industry practice despite its continued ineffectiveness as evidenced from recent *Escherichia coli* O157:H7 outbreaks, with irradiation now attracting increased attention as one means to assure end-product safety.

Purpose: Recognizing potential concerns surrounding cross-protection, this study assessed the ability of sanitizer-induced cell injury to enhance resistance of *E. coli* O157:H7 to X-ray irradiation on baby spinach.

Methods: A 3-strain *E. coli* O157:H7 cocktail (9.7 log CFU/ml) was exposed to two different sanitizers used in flume washing systems - 5.2 ppm peroxyacetic acid-based (PAB, Tsunami® 100) and 22 ppm chlorine-based (CB, XY-12®) sanitizer, and 18 ppm quaternary ammonium compound-based sanitizer used in equipment sanitation (QACB, Whisper™) to obtain 90 to 99% injury as determined by plating on Trypticase Soy Agar with 0.6% yeast extract (TSAYE) and Sorbitol MacConkey Agar (SMAC). Pre-irradiated, round-cut (5.1 cm^2) baby spinach leaves were then immersed for 5 min in the injured cocktail (resuspended to 8.5 log CFU/ml phosphate buffer), spin-dried, and irradiated in Whirl-Pak® bags at doses of up to 0.063 kGy (confirmed using radiochromic films) using a prototype low-energy X-ray irradiator (Rayfresh Foods, Ann Arbor, MI). Healthy and injured survivors were respectively quantified by plating appropriate dilutions on SMAC overlayed with TSAYE and SMAC after 48 h of incubation at 37°C.

Results: Results showed that *E. coli* O157:H7 injury on inoculated pre-irradiated spinach decreased from 90-99 to 66, 63, and 1% for PAB-, CB-, and QACB-treated cells, respectively. D_{10}-values for PAB-, QACB-, and CB-injured *E. coli* O157:H7 on baby spinach were 0.0136, 0.0223, and 0.0242 kGy, respectively. Prior exposure to PAB significantly ($P < 0.05$) enhanced *E. coli* O157:H7 susceptibility to X-ray irradiation, while exposure to CB significantly ($P < 0.05$) reduced susceptibility.

Significance: These results suggest that PAB sanitizers may be preferred for irradiated leafy greens since exposure to the CB sanitizer led to some cross-protection.

P3-130 *Salmonella* Transfer and Survival on Tomatoes and Contact Surfaces under Various Transportation and Storage Conditions

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Introduction: During transportation from the field to the market, tomatoes may become contaminated with pathogens, as they contact different surfaces. Since water influences pathogen transfer and survival, reducing moisture on tomato surfaces may reduce pathogen transfer.

Purpose: This study determined the rate of *Salmonella* transfer between tomatoes and contact surfaces at varying moisture conditions.

Methods: Tomatoes were spot inoculated with approximately 7 logs CFU rifampicin-resistant *Salmonella* and dried to 3 levels of dryness: wet with no drying time, 1 h of drying time and 24 h of drying time. Treated tomatoes were exposed to an 8 cm x 8 cm surface of a material commonly found in transportation containers and repacking facilities (HDPE, stainless steel, and vinyl) for four time periods at 25°C and 85% humidity: 0 (immediate touch), 1, 4 and 7 days. Pathogen transfer was evaluated following separation of the tomato and the surface by rinsing both with 20 mL of 0.1% peptone, and spiral-plating onto tryptic soy agar plus rifampicin (TSAR). TSAR agar plates were incubated at 37°C and surviving *Salmonella* enumerated after 24 h. Tomatoes inoculated and allowed to dry as described, but with no exposure to surfaces, served as controls.

Results: In control tomatoes with no exposure to surfaces, *Salmonella* levels decreased from 5.3 logs to 1.4 logs after 7 days. In experimental tomatoes held in contact for 7 days, 6.27 logs were collected from the tomato and 5.92 were collected from the surface. Wet contact immediately after inoculation resulted in the highest transfer rate. Transfer rates were lowest when inoculated tomatoes were dried for 24 h prior to contacting surfaces.

Significance: Allowing contaminated tomatoes to dry, prior to contacting surface materials, reduces initial *Salmonella* levels. Maintaining surface contact, over time, promotes survival but has little effect on transfer.

P3-131 Survival of *Salmonella* on Fresh Tomatoes under Selected Simulated Transportation Conditions

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Introduction: Fresh tomatoes contaminated with *Salmonella* have caused outbreaks of illness in consumers. Tomatoes can become contaminated in the field, during harvest and packaging, and in transit to or at the retail market. Tomatoes in transit to market are often subject to abusive temperatures that may enhance pathogen survival or proliferation.

Purpose: This study examined whether or not optimal and abusive temperatures for the storage of fresh tomatoes during refrigerated transport increased the ability of *Salmonella* to survive on tomatoes.

Methods: A cocktail of *Salmonella enterica* serovars associated with foodborne salmonellosis outbreaks (Michigan, Montevideo, Newport, Poona, and Saintpaul) were spot-inoculated onto the surfaces of mature green/breaker stage tomatoes (approximately 7 log CFU/tomato). Tomatoes were stored at temperatures of 7, 12, 15 and 25°C to simulate temperatures encountered during transportation to market. At sampling times, tomatoes were removed from storage and rinsed in 20 ml of 0.1% peptone. Rinses were diluted as needed and spread plated onto tryptic soy agar plus rifampicin (TSAR). Plates were incubated at 37°C for 24 h, and surviving *Salmonella* were enumerated.

Results: Overall, there was a decrease in *Salmonella* populations on the tomatoes over 72 h of storage. *Salmonella* survival was highest on tomatoes stored at 12°C and lowest on tomatoes stored at 7°C. However, 7°C is an abusive temperature at which to store mature green/breaker stage tomatoes in transport from the farm to the retail market as it results in chill injury. *Salmonella* survival on tomatoes stored at 15°C and 25°C was similar, however, 25°C is considered an abusively high, non-refrigerated temperature.

Significance: At optimal transportation temperatures for best quality, *Salmonella* populations survived but did not proliferate during a 72-h period.

P3-132 Impact of Post-inoculation Hold Time on *Escherichia coli* O157:H7 Transfer during Commercial Production of Fresh-cut Leafy Greens

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Introduction: Leafy greens are prone to pathogen contamination in the field as well as during subsequent handling and processing with the strength of bacterial attachment to the leaf surface being highly variable.

Purpose: Consequently, this study assessed the impact of post-inoculation hold-times and temperatures on *Escherichia coli* O157:H7 transfer during commercial lettuce processing.

Methods: Triplicate batches (0.5 kg) of Radicchio were dip-inoculated with a 4-strain cocktail of avirulent, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 (~10^5 CFU/g) and then processed either immediately or after storage for 1 d at 4°C or 5 d at 22°C. After priming the processing line that included a shredder, step conveyor, flume tank and shaker table with 2.3 kg of uninoculated iceberg lettuce, the inoculated Radicchio was processed, followed by 45.5 kg of uninoculated iceberg lettuce. Eight lettuce/Radicchio (25 g), 8 water (500 ml) and 50 equipment swab (100 cm^2) samples were collected during processing. After processing, all Radicchio pieces from eight 5-kg baskets of shredded lettuce, equipment and floor were collected and weighed after which three iceberg lettuce (25 g) samples from each basket were examined for *E. coli* O157:H7 by direct plating or membrane filtration using trypticase soy agar containing 0.6% yeast extract and 100 ppm ampicillin.

Results: After processing, *E. coli* O157:H7 was found in all iceberg lettuce samples with mean counts highest for 1 h- (0.28 log CFU/g) followed by 1 d- (-0.04 log CFU/g) and 5 d-held product (-0.40 log CFU/g) ($P < 0.05$) with scanning electron microscopy images showing greater bacterial attachment for 5 d-held product. *E. coli* O157:H7 populations in the flume water did not differ ($P > 0.05$) for 1 h (-1.23 log CFU/ml) compared to 1 d (-0.93 log CFU/ml) and 5 d-held product (-1.42 log CFU/ml) with a similar trend seen for the equipment surface samples.

Significance: Based on these findings, the point at which contamination occurs will impact the extent of cross-contamination during processing with significantly less *E. coli* O157:H7 transfer expected from field-contaminated leafy greens as opposed to product contaminated closer to the time of processing.

P3-133 Quantitative Transfer of *Escherichia coli* O157:H7 from Inoculated to Uninoculated Leafy Greens during Shaker Table Dewatering

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Introduction: Leafy greens are prone to cross-contamination during commercial shredding, conveying and flume washing with the potential for further spread of bacterial pathogens including *E. coli* O157:H7 via direct leaf-to-leaf contact during shaker table dewatering.

Purpose: Consequently, the goal of this study was to determine the rate of *E. coli* O157:H7 transfer between products during partial dewatering on a commercial leafy green shaker table.

Methods: In this study, one (0.03 g) or eight (0.38 g each) Radicchio pieces were manually cut from a single leaf, dip-inoculated to contain (~10^4 CFU/g) of a 4-strain avirulent, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 cocktail and dried for 1 h at 22°C. Thereafter, 300 or 297 g of uninoculated iceberg lettuce was uniformly cut, wetted, briefly drained, and placed into each of seven square metal baskets (12'' x 7'' x 7'') mounted to the side of a commercial-scale shaker table, followed by the addition of one (0.03 g) or eight (0.38 g each) pieces of inoculated Radicchio to the baskets while shaking to obtain inoculated to uninoculated (w/w) ratios of 1:100 and 1:10,000, respectively. The baskets were removed after 0 s, 10 s, 1 min, 5 min, 10 min, 30 min or 60 min. All shreds of Radicchio and two 25-g samples of only iceberg lettuce were then retrieved from the baskets. Sample homogenates were quantitatively examined for *E. coli* O157:H7 by direct plating w/o prior membrane filtration using trypticase soy agar containing 0.6% yeast extract and 100 ppm ampicillin as the growth medium.

Results: At the 1:10,000 ratio, *E. coli* O157:H7 transfer plateaued after ~22 min with average populations of 0.11 log CFU/g recovered from previously uninoculated iceberg lettuce. As expected, transfer leveled off faster for the 1:100 ratio (~0.5 min) with *E. coli* O157:H7 counts on the lettuce averaging ~2.52 log CFU/g.

Significance: These findings confirm that cross-contamination can occur via leaf-to-leaf contact with *E. coli* O157:H7-contaminated product within a very short time frame after a contamination event.

P3-134 Quantitative Transfer of *Salmonella* to Water and Equipment during Simulated Commercial Washing of Tomatoes

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Introduction: Post-harvest washing is recommended by the FDA to minimize microbial food safety hazards during tomato production. However, cross-contamination of tomatoes with *Salmonella* can occur during washing through contact with the wash water and equipment.

Purpose: This study quantified the transfer of *Salmonella* from dip-inoculated tomatoes to equipment surfaces and water during simulated commercial washing.

Methods: Triplicate 25-lb (11.3 kg) batches of greenhouse-grown red round tomatoes were dip-inoculated to contain *Salmonella* Typhimurium LT2 (avirulent) at 6 and 2 log CFU/g, air-dried for 2 h in a biosafety cabinet and then washed in sanitizer-free water for 2 min in a 3.3-m flume tank equipped with two overhead spray jets and dried on a 0.4-m x1.5-m polyethylene roller conveyor. During washing, two tomatoes (~450 g) and single water samples (50 ml) were collected at 15 sec intervals. After washing, eight flume tank (100 cm^2) and six roller conveyor (350 cm^2) surface samples were obtained using Kimwipes®. Tomatoes were hand-rubbed in 200 ml of phosphate buffer in a Whirl-pak bag for 2 min and then surface-plated on trypticase soy agar containing 0.6% yeast extract, 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate with or without 0.45 µm membrane filtration to quantify *Salmonella*. Surface samples were homogenized by stomaching in 15 ml of phosphate buffer for 1 min and then similarly examined for salmonellae along with the water samples.

Results: Midway through 2 min of washing, *Salmonella* populations stabilized at 4.12 and 0.53 log CFU/ml in the wash water for the high and low inocula, respectively, with populations decreasing 0.99 – 1.15 log CFU/g ($P > 0.05$) on the tomatoes. After washing, 0.01 – 0.02 and 0.001 – 0.03% of the original inoculum, respectively, transferred to the flume tank and roller conveyor surfaces. Roller-dried tomatoes retained 10.19 and 3.96% of their original high and low inocula, respectively.

Significance: This is the first report to quantitatively measure *Salmonella* transfer from tomatoes to equipment and water in a simulated commercial washing system. These findings, which clearly demonstrate how cross-contamination can occur during tomato washing, are critical to the development of science-based transfer models for risk analysis.

P3-135 *Listeria monocytogenes* Transfer during Mechanical Dicing of Celery and Growth during Subsequent Storage

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Introduction: During January to October 2010, 10 listeriosis cases, including 5 deaths, were traced to commercially diced celery from Texas.

Purpose: In response to this outbreak, *Listeria monocytogenes* transfer was assessed during mechanical dicing of celery along with growth of this pathogen in diced celery during 7 days of refrigerated storage.

Methods: In each of three experiments, 250 g of retail celery stalks were cut to 5-cm in length and inoculated by immersion for 1 h in an aqueous 5-strain *L. monocytogenes* cocktail containing 7.02 log CFU/ml to obtain 4.85 log CFU/g. After 30 min of drying at 22 °C, this batch of inoculated celery was diced using a Hand-Operated Easy Dicer Food Cutter Model N55100E (Nemco Inc., Hicksville, OH) followed by 15 identical 250-g batches of uninoculated celery. Each batch of diced celery was then transferred to a sterile Whirl-Pak bag and stored for 7 days at 4 °C, after which a 25-g sample was homogenized by stomaching, appropriately diluted and surface-plated with or without prior membrane filtration on Modified Oxford Agar to quantify *Listeria*. In addition, three 250-g celery samples were similarly inoculated at 4.81 log CFU/g, diced and examined daily for numbers of *Listeria* during 7 days of storage at 4 °C.

Results: Results showed that *L. monocytogenes* transferred from the initial batch of inoculated celery to all 15 batches of uninoculated celery during dicing with populations decreasing from 5.24 to 0.24 log CFU/g. The *Listeria* population averaged 2.29 CFU/g across all 15 batches with a 2-log decrease seen after dicing the first four 250-g batches. However, *Listeria* failed to grow in diced celery ($P < 0.05$) during the 7-day storage study.

Significance: Based on these findings, even though dicing can amplify an isolated contamination event, *L. monocytogenes* is unlikely to reach potentially hazardous levels in diced celery after one week of refrigerated storage.

P3-136 *Salmonella* Enteritidis Cross-contamination onto Mango by Means of Contaminated Knives

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Introduction: The possible transfer of *Salmonella* Enteritidis (SE) onto cut mango (*Mangifera indica* Linn.) due to contaminated knives may occur depending on the level of contamination of this utensil, but this hypothesis has not been demonstrated yet.

Purpose: To determine the cross-contamination of SE onto mango by using a contaminated knife with different levels of inocula.

Methods: Fruits without any defects (peel ruptures, bruised areas) were used in the experiment. Mangoes were aseptically surface disinfected with an alcoholic solution of iodine (BAM-FDA) and left 20 minutes in an air flow cabinet. Knives were previously inoculated on both sides with a final bacterial population of 10^6, 10^4 and 10^3 CFU in 100 ml of peptone water (0.1%), and left to dry inside the air cabinet. These contaminated knives were used to cut the mango. The cut pieces were placed on HE and XLD plates, with the cut side facing the agar surface, moved back and forth and then discarded. The occurrence of SE on each mango piece, following incubation at 35 °C/24 h, was determined by counting the colonies on HE and XLD. Bacterial colonies were confirmed by culture on TSI and LIA, and then biochemically confirmed using API 20E (biomérieux). Three repetitions for each essay were carried out. After cutting the mango, each knife was washed in 100 ml peptone water, and 1 ml of this water was pour plated in TSA. The plates were incubated at 35 °C for 24 h followed by counting, with the results being expressed in CFU/g.

Results: Detection of SE in cut mango was observed for knives inoculated at 10^6, 10^4 CFU/ml with this microorganism. Knife level contamination of 10^3 did not show any growth of SE on the cut fruit. Analysis of the washing knives did not show any colonies of SE.

Significance: The study indicates that the inside of a mango could be contaminated with *S.* Enteritidis during slicing by using a contaminated knife. Precautions should be taken in handling mango to minimize such contamination from the surface to the interior of the foods by using a cutting knife. Since SE can survive on the mango pulp even at lower temperatures good manufacturing practices should be applied for manipulation of this fruit.

P3-137 Evaluation of Factors That Impact Transfer of *Escherichia coli* from Gloves to Surfaces

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Introduction: Food safety concerns have lead to an increased use of gloves to hand-harvest fresh produce. However, little data exist to support development of appropriate management practices for their use.

Purpose: The purpose of this research was to evaluate factors that impact the transfer of *E. coli* from gloves to surface, including lettuce.

Methods: Latex or nitrile gloves were cut into 100 cm^2 pieces that were wrapped around a foam surface. Rifampicin-resistant *E. coli* was distributed onto the glove surface at a level of 2.5, 4.5 or 7.0 log CFU and tested immediately or allowed to dry for 30 min. Bacterial transfer was determined by placing the weighted inoculated gloves onto tryptic soy (TS) agar (TSA) plates or the surface of freshly-harvested Romaine lettuce leaves for 5 s. Lettuce leaves were homogenized in 0.1% peptone, the mixture filtered and plated onto TSA (limit of detection was 2 CFU/leaf) or were enriched in TS broth with rifampicin. Colonies were counted on TSA after incubation for 24 h at 35°C. Transfer coefficients were expressed as the number of *E. coli* transferred divided by the number of *E. coli* on the glove.

Results: In all cases, the transfer coefficient rapidly decreased over the first 10 sequential agar plates. Colonies were consistently observed over 20 plates at the 4.5 but not the 2.5 log CFU inoculum level. No significant difference ($P > 0.05$) in the transfer coefficient was observed between the 50- or 200-g weights. Transfer coefficients were significantly ($P > 0.05$) higher for the latex and dry inoculum than for the nitrile and wet inoculum. Log 2 CFU/leaf were transferred to each of six lettuce leaves at log 7.0 CFU/glove but transfer was not detected even by enrichment at log 4.5 CFU/glove.

Significance: Transfer of microorganisms from gloves to surfaces including lettuce is influenced by initial microbial loads reinforcing the importance of glove hygiene.

P3-138 Effect of Kitchen Procedures for Knife Cleaning on the Transfer of Pathogens during In-home Processing of Fresh Produce

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Introduction: Limited information exists describing either the transfer of pathogens from contaminated produce to other produce items during preparation of meals in the domestic environment, or the efficacy of cleaning procedures for inhibiting such cross-contamination.

Purpose: The objectives of this study were to determine the efficacy of methods for decontamination of knives used for consumer-mimicking processing of fresh produce and assess inhibition of produce cross-contamination via knives as affected by cleaning procedures.

Methods: Rifampicin-resistant *Salmonella enterica* (serovars Montevideo, Poona, Typhimurium) and *E. coli* O157:H7 were dip-inoculated onto waxed green bell peppers to 5.6 ± 0.5 $\log_{10}$ CFU/cm^2. Following drying, peppers were chopped into 1.0 cm^2 pieces and knives used to chop inoculated peppers were treated with either no treatment (control), wiped with a dry sterile cotton towel (TW), held under running warm water (60°C) for 5 or 10 s (5SW, 10SW), or sponge-scrubbed with a 1.0% (v/v) detergent solution and held under warm running water for 10 s (ST). Knives were then used to slice salad cucumbers into 2 mm wide slices. Transfer of pathogens from peppers to knives and from knives to cucumbers was determined by selective/differential enumeration of pathogens on lactose-sulfite-phenol red-rifampicin agar.

Results: Results indicate 5SW, 10SW, and ST treatments were nearly equally effective for decontaminating knives, with treatment-dependent reductions in *Salmonella* and *E. coli* O157:H7 ranging from 2.8–3.5 $\log_{10}$ CFU/cm^2. No differences ($P < 0.05$) were observed between mean numbers of pathogens on knives (1.2 $\log_{10}$ CFU/cm^2) and subsequent numbers of pathogens on cucumber slices (1.4 $\log_{10}$ CFU/cm^2), suggesting nearly all viable organisms remaining on knives after decontamination were transferred to cucumbers.

Significance: Findings suggest that produce-contaminated utensils can serve to contaminate other produce items during preparation of a meal, while proper decontamination can allow consumers to realize pathogen reductions in addition to those resulting from good agricultural practices and industry-applied interventions.

P3-139 Effects of Moisture Enhancement, Storage and Subsequent Cooking on Inactivation of *Campylobacter jejuni* and *Salmonella enterica* Typhimurium in Moisture-enhanced Pork

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Introduction: Translocation of bacterial pathogens into the interior tissue of pork meat through moisture enhancement may be of concern if enhanced pork is undercooked.

Purpose: The present study was conducted to evaluate the influence of moisture enhancement, storage temperature and cooking temperature on inactivation of *Salmonella enterica* Typhimurium and *Campylobacter jejuni* in moisture-enhanced pork during storage and cooking.

Methods: Boneless pork loins were surface inoculated with *Salmonella* Typhimurium or *Campylobacter jejuni* and injected with brine (sodium chloride, sodium phosphate) at 10% and 20%. Moisture-enhanced pork loins were sliced into 1cm thick slices and vacuum packaged at 4°C and 10°C. The slices stored at 4°C for 21 days and the slices stored at 10°C for 14 days were cooked to temperatures at the center of 155°F (68.3°C), 160°F (71.1°C), 165°F (73.9°C) and 170°F (76.7°C) on an electric grill, with holding for 0 min after cooking before excision of interior muscle from each slice and enumeration of *Salmonella* Typhimurium or *Campylobacter jejuni* from the interior muscle. A Generalized Linear Mixed Model (GLMM) using moisture enhancement, storage temperature, cooking temperature and different inoculated bacteria as independent variables, was developed to determine the influences of these variables on the probability of bacterial presence (the proportion of samples from which bacteria were recovered on the medium) in pork slices after grilling.

Results: Campylobacter jejuni was significantly less resistant to various treatments than *Salmonella* Typhimurium. Higher cooking temperature was more significantly effective for complete bacterial inactivation. Cooking above 160°F may be adequate for assuring the microbiological safety of moisture enhanced pork slice that is prepared without excessive contamination of interior tissues. Significant interactions between storage temperature and moisture enhancement level were observed.

Significance: No significant relationships existed between the probability of bacterial presence in pork slices after grilling and moisture-enhancement level. Moisture-enhanced pork does not present a greater risk to consumers than otherwise similar meat that is intact, provided that the meat is properly cooked.

P3-140 Combination of Slightly Acidic Low Concentration Electrolyzed Water with Calcium Lactate to Ensure Microbial Safety, Shelf Life and Sensory Quality of Fresh Pork

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Introduction: The consumption of pork products is increasing, so the microbial safety of pork during storage and marketing remains a concern. Constant efforts have been made to create effective and new technologies for the decontamination of carcasses and meat products.

Purpose: The objectives of this study were to evaluate the effectiveness of slightly-acidic low-concentration electrolyzed water (SlALcEW) and other carcass decontaminants against *Escherichia coli* O157:H7 and *Listeria monocytogenes* in fresh pork and to conduct the shelf life/sensory study of pork.

Methods: Samples of fresh pork were inoculated with approximately 5 log CFU/g of *E. coli* O157:H7 (B0259, B0297 and B0299) and *L. monocytogenes* (ATCC 19115, 19111 and Scott A) and dip-treated with distilled water (DW), aqueous ozone (AO), 1% lactic acid (LA), 1% calcium lactate (CaL), sodium hypochlorite solution (NaOCl), SlALcEW, StAEW, and SlALcEW + CaL for 5 min at room temperature (23 ± 2°C). Survival and growth of pathogens inoculated onto pork was also observed after treated with SlALcEW, StAEW and SlALcEW + CaL at 4, 10, 15, 20, 25, and 30°C. Each independent trial was replicated thrice and data for bacterial reduction was recorded as log CFU/g.

Results: Washing with sanitizers significantly ($P < 0.05$) reduced the inoculated pathogens compared to the unwashed control. The greatest reduction (3.0-3.2 log CFU/g) was achieved with SlALcEW + CaL against *E. coli* O157:H7 and *L. monocytogenes* and there was no significant difference ($P > 0.05$) found between SlALcEW and StAEW treatment. Compared with unwashed control we found that treated with SlALcEW + CaL extended the shelf life of pork up to 6 days at 4 °C storage with little change in color and odor.

Significance: The slightly-acidic low-concentration electrolyzed water is novel, and no studies examine the effect of SlALcEW and its combination with calcium lactate to decontaminate fresh pork. The results of the current study are very promising, although carried out in laboratory conditions.

P3-141 Efficacy of a Food-grade Blend of Lactate-diacetate-propionate as Ingredients to Control *Listeria monocytogenes* on Commercially-produced Frankfurters

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Introduction: Further research is warranted to evaluate different levels/types of food grade antimicrobials to control *Listeria monocytogenes* (Lm) on RTE meats.

Purpose: Determine viability of Lm on frankfurters formulated with and without a blend of lactate-diacetate-propionate (0.0%, 0.5%, 0.75%, or 1.0%) and then surface treated with lauric arginate (LAE; 22 ppm) or levulinic acid (LEV; 160 ppm) and stored at 4°C for up to 90 days.

Methods: Each package of 8 links (ca. 454 grams) was surface inoculated with ca. 4.3 log CFU/package of a five-strain mixture of Lm. Following an attachment period, 3 ml of LAE or LEV were delivered into each package using the Sprayed Lethality in Container (SLIC®) delivery method. The pathogen was enumerated throughout storage using the USDA package rinse/recovery method.

Results: Without antimicrobials, Lm increased from ca. 4.3 to ca. 7.7 log CFU/package after 90 d. When frankfurters were formulated with 0.5% of the blend, pathogen numbers remained relatively unchanged over 75 d, but then increased to ca. 5.8 log CFU/package after 90 d. When 0.75 or 1.0% of the blend was added, Lm remained relatively unchanged or decreased somewhat after 90 d. Regardless of whether the blend was included, when frankfurters were surface treated with LAE or LEV, Lm decreased from ca. 4.3 to ca. 1.5 to 2.5 log CFU/package within 2 h. However, after 90 d Lm increased to ca. 9.0 and 5.8, 5.8 and 5.6, 2.6 and 4.0 log CFU/package on frankfurters formulated with 0.0%, 0.5%, or 1.0% of the blend and treated with LAE and LEV, respectively, whereas numbers decreased to ca. 0.8 and 2.5 log CFU/package, respectively, using 0.75% of the blend.

Significance: Inclusion of lactate-diacetate-propionate as ingredients, with or without LAE or LEV on the surface, can control Lm on frankfurters throughout shelf life.

P3-142 Use of Enhanced Octanoic Acid Surfactant System as a Post-lethality Treatment to Reduce *Listeria monocytogenes* on Hot Dogs

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Introduction: It is estimated that over 1,500 cases of Listeriosis occur each year in the United States. Both FSIS and FDA identify hotdogs as posing a relatively high-risk for illness from *Listeria monocytogenes*. Currently, octanoic acid (OA) can be used as a post-lethality treatment (PLT) that meets the requirements of Alternative 1 or Alternative 2 determined by FSIS for the safety of ready-to-eat (RTE) meats. Historically it has been difficult to treat hot dogs with an effective in-package PLT while maintaining low volumes of purge in the finished package. Development of a hot dog PLT that results in a 1–2 log reduction in *Listeria monocytogenes* with a treatment volume of 3.5 mL or less is therefore highly desirable.

Purpose: The purpose of this study was to test the efficacy of an enhanced OA treatment of hot dogs inoculated with *L. monocytogenes*. The OA solutions used were solubilized using either a blend of food grade, GRAS surfactants (OA-B) or a single surfactant system (OA-S). Additionally, the effect of OA-B treatment on *Listeria* populations during long term refrigerated storage of these products was observed.

Methods: Hot dogs were inoculated with a five-strain cocktail of *L. monocytogenes*. An in-package application of 3.5 mL of OA treatment solution (OA-B or OA-S) was used on each sample (10 hot dogs arranged in two layers with a total package weight of 12 oz). Each package was treated just prior to vacuum sealing. The concentration of OA in either surfactant system was adjusted to two residual octanoic acid levels (46 and 92 ppm residual OA by weight of the packaged hot dogs).

Results: Treatment of hot dogs with OA-B at 46 and 92 ppm residual OA by weight of the packaged hot dogs yielded a reduction of 1.5 and 2.0 log CFU/package respectively, after 24 hours storage at 4°C. This level of inactivation was greater by ~1.0 log CFU/package than that achieved using OA-S at the same levels. Levels of *L. monocytogenes* on hot dogs were maintained at <2 log CFU/package over a four week storage period at 4°C.

Significance: Results demonstrate the effectiveness of a blended surfactant system (OA-B) treatment to meet FSIS guidelines for a post-lethality treatment for RTE hot dogs as well as meeting hot dog processor requirements of low package purge volumes.

P3-143 Evaluation of Process Parameters Used during the Fermentation and Drying of Italian-style Salami

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Introduction: Fermented meat products such as Italian dry salami continue to be associated with outbreaks of foodborne illness. These ready-to-eat products and their specialized processes rely on factors such as low water activity, low pH, salt and/or combinations of these factors to provide microbial stability and to ensure their safety.

Purpose: To evaluate the reduction of a five strain cocktail of *Salmonella* spp. subjected to three different drying processes applied following fermentation during the production of Italian dry salami.

Methods: Five strains of *Salmonella* spp. isolated from low water activity foods were used in combination to inoculate raw ground pork. The salami batter was prepared according to a commercial manufacturing recipe and processing directions. Following the addition of non-meat ingredients, the batter was stuffed into 100 mm diameter collagen casings. Ten sticks were placed into three environmental chambers for fermentation (total n = 30). Following fermentation, each chamber was set at one of three different temperatures (low, medium, high) and dried to a water activity of ≤ 0.92. Core samples were collected from salami sticks at scheduled intervals throughout fermentation and drying and analyzed for levels of lactic acid starter culture, *Salmonella*, pH, and water activity. Levels of *Salmonella* in samples taken during the process were determined using serial dilutions and enumeration plating on selective media.

Results: The fermentation process resulted in approximately a 2.7 to 3.3-log reduction in levels of *Salmonella*. An additional 1.4 to 3.0-log reduction was reported during drying for a total log reduction of 5.1, 6.1 and 6.3 for the low, medium and high drying temperature treatments, respectively. Based on these results the total reduction in levels of *Salmonella* was the result of both the fermentation and drying processes.

Significance: These results emphasize the importance of validating the individual as well as the combined effect that both fermentation and drying contribute to the process and overall reduction in levels of *Salmonella* in these unique types of products and to quantitatively assess processes that are often described to be as much of an art as a science.

P3-144 Antimicrobial Activity of Lactic Acid Bacteria against *Listeria monocytogenes* on Ready-to-Eat Meat

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Introduction: Contamination of *Listeria monocytogenes* has been a constant threat for the deli meat industry due to the high mortality rate. Lactic acid bacteria (LAB) have shown protective action against various pathogenic bacteria. *Pediococcus acidilactici, Lactobacillus amylovorus* and *Lb. animalis* were previously isolated from commercial ready-to-eat (RTE) meat.

Purpose: The aim of this study was to evaluate the anti-listerial activity of the combined three LAB strains on *L. monocytogenes*.

Methods: A spot-on-lawn assay was performed with the combined LAB and showed a zone of inhibition with 1.29 ± 0.09 mm against the cocktail of *L. monocytogenes* after 24 h. The zone was observed up to 1:128 dilutions with 0.72 ± 0.04 mm. *P. acidilactici* showed inhibition with 1.22 ± 0.06 mm. Competitive growth assay in TSBYE was performed by adding different concentrations of LAB ranging from 10^{10} CFU/ml to zero cells with the presence of 10^3 CFU/ml of *L. monocytogenes* at 7 and 4°C for 3 and 7 days.

Results: LAB with higher than 10^8 CFU/ml had bactericidal effect by 1-2.5 log reduction of *L. monocytogenes* at both refrigerated and temperature-abused conditions. However, a 1-1.8 log increase of viable *L. monocytogenes* was observed with lower than 10^7 CFU/ml of LAB after one week. Although overall concentration of *L. monocytogenes* was higher with 0.2–0.5 log when stored at 7°C than refrigerated temperature, combined LAB showed similar inhibition pattern to *L. monocytogenes* at both temperatures.

Significance: Overall, three combined LAB strains may be suitable to prevent or to minimize growth of *L. monocytogenes* for the potential application in RTE meat products.

P3-145 Viability of *Listeria monocytogenes* on Commercially Prepared, Uncured Turkey Breast, Formulated with and without Potassium Levulinate, Potassium Diacetate and Potassium Propionate during Extended Refrigerated Storage

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Introduction: Although *Listeria monocytogenes* (Lm) is eliminated from ready-to-eat (RTE) red meat and poultry products during thermal processing, it can be reintroduced onto the surface of such products via post-process contamination. Thus, further research is warranted to evaluate the efficacy of food grade antimicrobials for suppressing outgrowth of Lm on RTE meats during shelf life.

Purpose: Evaluate the efficacy of potassium levulinate, diacetate, and propionate to inhibit Lm on commercially-prepared, uncured turkey breast during refrigerated storage.

Methods: Formed, uncured turkey breast (ca. 5 kg each) was formulated with or without potassium levulinate (1.0, 1.25, 1.5, 1.75, and 2.0%), alone or in combination with potassium diacetate (0.1%) and potassium propionate (0.1%), by a commercial processor. Finished product was sliced (ca. 1.25 cm thick) and subsequently surface inoculated on both the top and bottom faces to a target level of ca. 3.5 log CFU/slice with a five-strain cocktail of Lm. The inoculated slices were placed into nylon-polyethylene bags, vacuum-sealed, and stored at 4°C for up to 90 d. The pathogen was enumerated throughout storage using the USDA package rinse/recovery method.

Results: Without inclusion of any antimicrobials in the formulation, Lm numbers increased by ca. 5.2 log CFU/slice, whereas with the inclusion of 1 to 2% of levulinate in the formulation, pathogen numbers increased by ca. 0.2 to 2.7 log CFU/slice over 90 d at 4°C. In contrast, a synergistic effect was observed when levulinate was added in combination with 0.1% diacetate and 0.1% propionate. When all three antimicrobials were included as ingredients, pathogen numbers decreased by 0.3 to 0.6 log CFU/slice after storage at 4°C for 90 d.

Significance: Our results validate that levulinate, alone or in combination with diacetate and propionate, would be effective as an ingredient for inhibiting outgrowth of Lm during refrigerated storage of uncured turkey breast.

P3-146 The Use of a Bread Proofer in Deli Environments to Aid in the Thermal Inactivation of *Listeria monocytogenes* on Deli Slicers

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Introduction: Some commercial delicatessen environments have been reported to harbor a variety of foodborne pathogens including *Listeria monocytogenes* (Lm). This organism persists in niches in the deli environment because of its ability to survive in cold, wet environments and to exist in protective biofilms. Some strains of Lm are more resistant to thermal inactivation and a range of sanitizers. Even though a majority of commercially produced deli meats have been properly manufactured and thermally processed to eliminate Lm, post-processing contamination can occur. The Food Safety and Inspection Service (FSIS) and the United States Department of Agriculture (USDA) in 2009 stated that one of the greatest risks of Lm foodborne contamination is from deli meats that are sliced in commercial delis.

Purpose: Some commercial operations use dry heat because they believe it will kill Lm on food equipment. Tests in our laboratory determined that placing a deli slicer in dry oven conditions at 82°C for 15 hours did not sufficiently decrease Lm when it was inoculated onto deli slicer components. However, further studies determined that moist heat, relative humidity > 90%, at 85°C for 3 hours achieved a 4.5 to 5 log reduction of Lm. Our objective was to bring these conditions to the deli environment by utilizing the bread proofer that is normally present in delis.

Methods: We placed 100 mL of distilled water in the bottom compartment of the bread proofer and ran it on "proofer" mode at 68°C for 7 hours. Dry heat followed the moist heating step to prevent damage to the motor and other moving parts of the deli slicer.

Results: The interior conditions of the proofer reached 95% relative humidity for 45 min, decreasing to 35% and lower for the duration. These conditions resulted in an approximate 5 log reduction of Lm.

Significance: This research presents a useful protocol to improve food safety in the deli environment.

P3-147 Poultry Contamination with *Salmonella* and *Campylobacter* from Farm to Slaughter

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Introduction: Salmonella and *Campylobacter* have long been recognized as important pathogens in poultry meat. In the USA, inspection procedures based on HACCP include *Salmonella* and *E. coli* monitoring and zero tolerance for fecal contamination. In the European Union, *Salmonella* and *Campylobacter* are evaluated in chicken carcasses before and during slaughter. In Brazil, a *Salmonella* reduction program started in 2003 on poultry carcasses. Fecal contamination on carcasses before chilling is considered a critical control point (CCP) compulsory for the slaughtering of poultry.

Purpose: The purpose of this study was to determine the presence of *Salmonella* and *Campylobacter* before slaughter on the farm and also associate with intestine contamination and carcasses with different fecal contamination during the slaughter in industries in which there is a CCP compulsory for slaughtering of poultry in Brazil.

Methods: This study was conducted in a commercial plant located in the South of Brazil that processed six-week-old chicken under industrial conditions. On the farm, *Salmonella* (n=40) and *Campylobacter* (n=17) flocks were sampled for analysis with drag swabs. During slaughter, the intestinal content of previously analyzed flocks and carcasses with and without fecal contamination and carcasses after removal of fecal contamination by trimming from the same flocks were analyzed. The samples were analyzed with BAX system Q7 and confirmed with bacteriological assay.

Results: Salmonella was present in 5% of chickens at farms before slaughter. *Salmonella* Agona and *S.* Lexington were identified in the positive samples. During slaughter, *Salmonella* was not detected in the intestine and in the carcasses with and without fecal contamination and in the carcasses after the fecal contamination was removed by trimming. Unlike *Salmonella, Campylobacter* was positive in all farms and intestine samples. Contamination with *Campylobacter* was observed in 76.5% of the carcasses with fecal contamination, 27.5% of the carcasses without contamination and 31.4% after removal of the contamination by trimming.

Significance: The results indicate that the measures which were effective for controlling *Salmonella* in the poultry chain were not effective for controlling *Campylobacter*. The presence of feces in poultry carcasses was not associated with the presence of *Salmonella*, but it increased *Campylobacter* contamination. The control of *Salmonella* and *Campylobacter* contamination on chicken flocks before slaughtering is a control measure during slaughtering so as to prevent cross contamination and protect the meat from food safety problems.

P3-148 Isolation and Characterization of Avian Pathogenic *Escherichia coli* from Delmarva Poultry

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Introduction: Avian pathogenic *E. coli* (APEC) isolates are widespread in poultry flocks and cause colibacillosis in birds, resulting in severe economic losses every year in the U.S. APEC strains are closely related to human uropathogenic *E. coli* (UPEC), and there have been recent reports linking APEC with human diseases and the finding of *stx1* and 2 genes in APEC may increase this risk. Because APEC isolates can be isolated from a range of retail foods and have the potential to be a zoonotic risk, there is a growing concern of APEC contamination in our food supply.

Purpose: To characterize APEC strains isolated from poultry flocks in Delaware and better understand their role in causing foodborne diseases and acting as a zoonotic agent.

Methods: APEC strains were recovered from broilers which showed clinical symptoms of *E. coli* infection and grown on Tryptic soy agar (TSA) plates. After confirmation using selective media, APEC strains were serotyped, and selected O antigen strains (O157, O8, and O35) were plated on TSA and O157 strains were transferred onto SMA agar and screened for shiga toxin production. Colonies were picked from TSA and PCR was used to screen for *stxI, stxII* and *eae* genes.

Results: Three-hundred and fifty APEC strains were isolated from poultry flocks in Delmarva. Serotying showed that the majority of strains were O8, O35 and O78, and only eight strains were O157. All eight O157 strains produced pink colonies on SMA agar and PCR tests were negative for *stxII* and *eae* genes. Further PCR tests will be conducted on all O type strains to determine the presence of other important virulence genes.

Significance: To determine the potential of APEC to become a zoonotic risk by testing for the presence of significant virulence genes.

P3-149 Spray Washing, Absorbent Corn Starch Powder and Dry Time to Reduce Bacterial Numbers on Soiled Broiler Transport Cage Flooring

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Introduction: Most broilers in the U.S. are transported live to slaughter facilities in cages with fiberglass floors. Cages are often used repeatedly without washing and fecal matter deposited on the floor surface can transfer *Campylobacter* from one flock to another. Drying feces out between uses is an effective but slow and logistically impractical means to kill *Campylobacter* in soiled transport cages.

Purpose: The objective of this study was to test the addition of an absorbent powder to boost efficiency of cage drying as a sanitation procedure.

Methods: Squares (5 X 5 cm) of fiberglass flooring were covered with broiler gut contents for 60 minutes, spray washed and then covered with a known quantity of corn starch or left untreated as controls. All squares were left to dry for 0.25, 2, 4 or 24 hours. Sterile sponges used to sample the surfaces and were stomached in PBS which was subsequently plated on campy cefex agar for *Campylobacter* counts, reported as log CFU per floor square.

Results: At 0.25 hours dry time, corn starch alone did not significantly lessen the number of *Campylobacter* compared to unwashed control (5.84 and 5.74 respectively); washing lowered numbers by more than one log (4.12) and washing followed by cornstarch lowered *Campylobacter* numbers by three logs (2.79). By two hours dry time, no *Campylobacter* was detected on spray washed flooring with or without corn starch compared to 5.60 on control unwashed and 4.46 on corn starch treated, unwashed flooring. At 24 hours dry time, no *Campylobacter* was detected on any flooring.

Significance: These data show that an absorbent powder may aid in the desiccation-caused death of *Campylobacter* on soiled transport cage flooring in a short turn around time scenario.

P3-150 Poultry Litter from Free-range (Organic) Chickens Harbor Multi-drug Resistant and Pathogenic Strains of *Salmonella*

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Introduction: Fresh and processed poultry has been implicated in several cases of salmonellosis. While previous studies have focused on *Salmonella* prevalence in commercially poultry production, there is limited information about the microbial genetics of these pathogens when birds are raised free range. Poultry litter is a major source of *Salmonella* contamination in pre-harvest poultry.

Purpose: This study evaluates the dynamics of *Salmonella* colonization in free-range and commercial chickens.

Methods: Salmonella isolates (n=128) from free-range and commercial chicken litter samples were characterized by antimicrobial susceptibility testing (AST) to 15 drugs, XbaI-PFGE restriction and plasmid (conventional and replicon typing) profiles. Multiplex PCR reactions were performed to detect 35 virulence genes related to *Salmonella* pathogenicity.

Results: Nearly, 83% (106/128) of the isolates were resistant to at least one of the antimicrobials tested, and 22% were resistant to five or more antimicrobials. The most resistance was shown to sulfisoxazole (66%), ampicillin (54%), streptomycin (46%), tetracycline (45%), ceftiofur (40%), cefoxitin (39%), and amoxicillin/clavulanic acid (38%). The PFGE dendrogram revealed 6 distinct groups with 80 to 90% similarity in their fingerprint profiles, and two groups with 50 to 60% similarity. The DNA band patterns ranged from 20 to 1000 kb. Plasmid profiling revealed that *Salmonella* isolates harbored 3 to 10 plasmids, ranging from 2 to 120 kb. Plasmid sizes of ~2, 4 or 120 kb were most frequently detected. The major replicon types identified were IncA/C, FIIA, FIB, IL, N or HI1. Plasmids from these replicon types are known to carry genes for antimicrobial resistance, virulence and/or disinfectant resistance. Nearly 96% (123/128) of isolates harbored >50% of the virulent gene determinants. The *virB4, virD4* and *cdtB* genes were not detected in any isolates, while *invA* and *iacP* were detected in all isolates.

Significance: Litter appears to be a major reservoir of multi-drug resistant, pathogenic, and genetically-diverse *Salmonella* populations. These pathogens harbor diverse replicon plasmids that may contribute to transfer of genes among the bacteria present in the environment and the birds.

P3-151 Growth Kinetics of *Salmonella* Enteritidis in Raw Ground Chicken with Different Levels of Native Microflora

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Introduction: Salmonella Enteritidis often contaminates chicken and eggs and has caused serious food poisoning outbreaks worldwide. Few researchers have studied growth kinetics of the pathogen and native microflora (NM) in ground chicken at various temperature patterns.

Purpose: The purpose of this study was to analyze and predict growth of *Salmonella* Enteritidis and NM in raw ground chicken with our mathematical model.

Methods: Commercial ground chicken with high (10^7 CFU/g) and low (10^5 CFU/g) levels of NM was injected with *Salmonella* Enteritidis strain 04-137 ($10^{3.2}$ CFU/g) and then stored at constant and dynamic temperatures ranging from 8 to 32°C. Bacteria counts of the pathogen and NM were measured with XLD agar plates and standard method agar plates, respectively. Data were analyzed with the model.

Results: Values of the maximum population, N_{max}, and the rate constant of growth, r, for *Salmonella* were higher at higher temperatures in chicken with both NM levels. N_{max} for NM was constant ($10^{9.4}$ CFU/g) at all temperatures for both NM levels. *Salmonella* growth, characterized by both N_{max} and r, was highest in sterilized chicken, followed by chicken with the low NM level and then chicken with the high NM level. With data at constant temperatures, growth of *Salmonella* and NM in chicken stored at dynamic temperatures was well predicted with the model; averages of standard error for *Salmonella* were low, being 0.049 ± 0.021 and 0.18 ± 0.059 log units in low and high NM chicken, respectively.

Significance: Our study clarified the effects of NM at different doses in ground chicken on growth kinetics of the *Salmonella* Enteritidis strain and the usability of the growth model for foods with NM.

P3-152 Prevalence of the Foodborne Pathogen *Campylobacter* spp. in Retail Meats in Tulsa, Oklahoma

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Introduction: Campylobacter is a foodborne pathogen that is considered as the second leading cause of bacterial illness in the U.S. *Campylobacter* spp. are found in the environment and as part of the natural intestinal flora of birds. Contaminated and undercooked meat is an important transmission vehicle for human *Campylobacter* infection.

Purpose: The purpose of this study was to isolate *Campylobacter* spp. from retail meats and to determine its prevalence in different types of meat such as chicken, turkey, pork, beef (including beef livers), and chicken livers and gizzards.

Methods: Retail meats were purchased and rinsed with BPW which was then inoculated into 2 x Bolton enrichment broths. The enrichment was then streaked onto CCDA *Campylobacter*-specific agar media and incubated for 48 h under microaerophilic conditions. Suspected *Campylobacter* colonies were picked up and subjected to identification using a multiplex PCR.

Results: A total of 611 samples of meat were purchased from several Tulsa area grocery stores and used for the isolation of *Campylobacter*. Of these, 231 (37.8%) were positive for *Campylobacter*. From the 231 positive samples, 130 (21.3%) were identified as *Campylobacter jejuni* and 108 (17.7%) as *Campylobacter coli* (a few samples contained both species). *Campylobacter* positive samples were distributed as follows: 134 (58%) from chicken livers and gizzards, 56 (24.2%) from poultry meat, and 41 (17.7%) from beef and pork.

Significance: Retail meat products in grocery stores carry *Campylobacter* species and proper cooking and handling methods need to be used to prevent foodborne illnesses. Chicken and beef livers appear as a potential risk factor for *Campylobacter* due to the high prevalence of this foodborne bacterium in those samples.

P3-153 Contamination of Individual Whole Broiler Chickens with Multiple Molecular Subtypes of *Salmonella* and *Campylobacter*

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Introduction: Outbreaks of human salmonellosis and campylobacteriosis are often associated with food and can be linked by molecular subtyping to poultry contaminated with *Salmonella* and *Campylobacter*. Knowledge of the frequency with which individual chickens are contaminated with multiple bacterial species/subtypes is imperative for optimizing the sampling strategy used to establish linkages between contaminated poultry and human disease.

Purpose: This study aimed to determine the prevalence and diversity of these pathogens in chickens from retail markets in Massachusetts, the frequency with which multiple contaminants are present in single birds, and which anatomic locations yielded the greatest diversity of contaminating species/subtypes.

Methods: Between October 2010 and April 2011, raw whole broiler chickens purchased in Massachusetts were tested for *Salmonella* sp., and *Campylobacter* sp. using FDA Bacteriological Analytical Manual protocols. The chickens were sampled from several anatomic locations and a whole carcass wash. Molecular subtyping was performed by pulsed-field gel electrophoresis as per Centers of Disease Control and Prevention PulseNet protocols.

Results: All 9 chicken carcasses were positive for *Salmonella* and *Campylobacter*, with as many as 7 *Salmonella* molecular subtypes and 13 *Campylobacter* molecular subtypes isolated from a single chicken. 51 (52.0%) of 98 sub-samples from various anatomic locations were positive for *Salmonella* and 53 (54.6%) of 97 sub-samples were positive for *Campylobacter*. In order to maximize the molecular subtypes found in each chicken, multiple anatomic locations would need to be sampled.

Significance: Contamination of poultry with multiple *Salmonella* and *Campylobacter* subtypes is common. Sampling multiple locations from each chicken, in addition to the whole carcass wash, may be necessary to yield the isolate(s) that can be linked by molecular subtyping to human disease. The frequency and biodiversity of contamination by human bacterial pathogens found on chickens will likely be underestimated in studies that are based on sampling from only a whole carcass wash or a limited number of locations.

P3-154 Comparison of Cumulative Drip Sampling to Whole Carcass Rinses for Estimation of *Campylobacter* spp. and Quality Indicator Organisms from Processed Broiler Chickens

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Introduction: The whole carcass rinse (WCR) procedure is routinely used as a sampling method for determining the presence and number of quality-indicator organisms and pathogens associated with broiler chicken carcasses in processing facilities. Collection of a cumulative drip sample by placing collection vessels under the processing could reflect a potentially more representative sample of bacterial populations associated with an entire flock with less labor than individual bird rinses.

Purpose: The purpose of this study was to evaluate a cumulative drip sampling method for recovery of *Campylobacter* spp. and three types of quality indicator organisms from broiler carcasses.

Methods: Samples were collected on 12 days from commercial broiler processing facility over a three-month period. WCR samples were obtained post-evisceration (PEWCR) and post-chill (PCWCR) and drip samples were obtained by placing multiple collection vessels at appropriate points post-evisceration, pre-chill, and post-chill on each collection day. *Campylobacter* spp. were counted by traditional direct plating techniques. Total viable count, *Enterobacteriaceae*, and *E. coli* populations were determined using the Tempo automated MPN procedure.

Results: Cumulative drip sampling was validated by demonstrating no statistically significant difference between the WCR and cumulative drip sampling methods in recovery of *Campylobacter* spp., total aerobes, *Enterobacteriaceae* or *E. coli* associated with the post-evisceration samples ($P > 0.2$). There was no significant correlation between any of the indicator organisms and *Campylobacter* spp. As expected, post-evisceration samples had greater numbers of bacteria associated with them than did post-chill samples. *Campylobacter* spp. (PCWCR mean Log 0.21) were more drastically reduced post-chill than the indicator organisms tested (PCWCR mean log 1.97), suggesting that in-plant intervention steps in place for *Salmonella* reduction are effectively reducing *Campylobacter* spp. populations as well.

Significance: A simple cumulative drip sampling technique may be useful in providing a representative summary of process control in poultry processing facilities.

P3-155 Pilot-scale Validation of a *Salmonella* Thermal Inactivation Model Applied to Whole-muscle Meat and Poultry Products Cooked in a Moist-air Impingement Oven

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Introduction: Microbial inactivation models can be used to evaluate the lethality of meat cooking processes. Typically, such models are based on data from laboratory-scale experiments and are rarely validated for industrial application.

Purpose: The objective of this study was to quantify accuracy and uncertainty of *Salmonella* inactivation models applied to whole-muscle meat products, via pilot-scale tests in a moist-air impingement cooking system.

Methods: Whole-muscle beef (round), pork (loin), or chicken (breast) samples (88 mm dia., 11 mm thick) were inoculated via vacuum submersion in a salt-phosphate marinade containing an eight-serovar *Salmonella* cocktail. Inoculated samples containing >7 log(CFU/g) were cooked in a pilot-scale, moist-air impingement oven under commercially relevant conditions. Process variables included humidity (20 or 50% moisture by volume), air temperature (149 or 204°C), and fan speed (2.7 m/s), with triplicate samples processed at each condition. Internal and surface temperatures were recorded real-time during processing. *Salmonella* inactivation was calculated by previously reported activation models (i.e., product-specific D and z values). Samples were removed from the oven at a target computed lethality (~2-4 log), rapidly cooled to an internal temperature of 50°C in liquid nitrogen and then immediately sliced into three layers, with a core (~50 mm diam, ~4 mm thick) removed from the center layer. All samples were stomached, serially diluted, and plated on modified tryptic soy agar plates (37°C, 48 h) to enumerate surviving salmonellae. Lethality error was defined as the difference between the experimental and predicted log reductions.

Results: The root mean squared errors for the computed lethalities for whole-muscle beef, pork, and chicken were 1.30, 1.54, and 1.43 log CFU/g, respectively. Mean errors (biases) were -0.13, 0.18, and -0.72 for whole muscle beef, pork, and chicken, respectively.

Significance: Therefore, such models should be validated for each specific product and application and should account for the inherent uncertainty when applied to industrial systems.

P3-156 A Universal Thermal Inactivation Model for *Salmonella* in Meat and Poultry Products

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Introduction: To validate *Salmonella* lethality in fully-cooked meat and poultry products, manufacturers may use pre-established time/temperature treatments (i.e., safe harbors) or mathematical inactivation models. The latter allows for greater process flexibility. However, available models are based on data from individual laboratories, and rarely account for the variety of factors relevant to industrial processes, or the expected uncertainty inherent in applying these models to independent processes.

Purpose: Therefore, the objective was to develop a universal thermal inactivation model for *Salmonella*, based on multiple sources of laboratory-based data, and to evaluate its performance against independent pilot-scale data.

Methods: Thermal inactivation data were gathered from ComBase and scientific literature. Data for *Salmonella* inactivation in turkey, beef, and pork (n = 353, 569, and 341, respectively) was highly variable across source studies, due to different sample preparation, processing, and recovery methods. To account for this, model prediction intervals (PIs) contained a random effect on the parameter with variance error. The fitting model, which was 1st-order/modified-Arrhenius, accounted for temperature, muscle type, and fat percentage for each species.

Results: The root-mean-squared errors for the model fitting were 0.41, 0.75, and 0.59 log CFU/g for turkey, beef, and pork, respectively. Values for the variance error parameter were 1.58 ± 0.94, 0.95 ± 0.75, and 0.74 ± 0.47 min-1, for the same species. When validated against pilot-scale data, the ground beef model yielded the best prediction, with 17 of 19 data points captured within the PIs. Mean residuals for turkey, beef, and pork, were -2.03, 0.65, and 1.11 log CFU/g, respectively.

Significance: Model performance was mostly affected by the nature of the raw data; variability in experimental methods across studies appeared to cause the PIs to be particularly large. This shows the need for standard methods for generating inactivation data, models, and parameters, in order to consolidate information across studies and increase collaboration to develop better prediction tools.

P3-157 Antilisterial Properties of Marinades against Post-cooking Inoculated Chicken Breast Meat during Refrigerated Storage and Microwave Oven Reheating

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Introduction: Refrigerated storage of cooked leftover chicken meat could potentially allow multiplication of *Listeria monocytogenes*, accidentally introduced after cooking, due to reduced levels of background microflora on cooked meat. Marination of chicken meat, in addition to adding value and improving palatability, may enhance the safety of refrigerated leftovers through the antimicrobial properties of the marinade ingredients.

Purpose: This study evaluated growth of *L. monocytogenes* inoculated on cooked, marinated chicken meat and stored aerobically at 7°C. Pathogen survival during microwave oven reheating of stored chicken was also investigated.

Methods: Raw chicken breast meat portions (100-g) were marinated (1 [meat]:2 [marinade] ratio), for 30 min at 4°C, with commercially formulated tomato-, soy-, and lemon-based marinades as well as tomato juice, soy sauce, and lemon juice. After marination, samples were cooked by pan-broiling to an internal temperature of 73.8°C, then cooled to 4°C and surface-inoculated with *L. monocytogenes* (5-strain mixture; 2.5 log CFU/g). Along with non-marinated controls, samples were stored at 7°C (up to 7 days) in Pyrex dishes overwrapped with plastic film, to simulate home refrigeration of cooked leftovers. On Days 0, 1, 2, 4, and 7 of storage, samples were reheated in a microwave oven (1100 W) for 45 or 90 s, and analyzed (two repetitions, three samples/treatment/repetition) for microbial counts, pH, and water activity. Data were statistically analyzed as a randomized complete block design.

Results: L. monocytogenes counts on non-marinated (control) samples increased ($P < 0.05$) from 2.7 ± 0.1 (Day-0) to 6.9 ± 0.1 (Day-7) log CFU/g during storage. Initial (Day-0) pathogen counts of marinated samples were < 0.5 log CFU/g lower than those of the control, irrespective of marinade treatment. At 7 days of storage, pathogen levels on samples marinated with tomato juice were not different ($P \geq 0.05$; 6.9±0.1 log CFU/g) from those of the control, whereas for samples treated with the remaining marinades, pathogen counts were 0.7 (soy sauce) to 2.0 (lemon juice) log CFU/g lower ($P < 0.05$) than those of the control. Microwave oven reheating reduced *L. monocytogenes* counts by 1.9 to 4.1 (45 s) and >2.4 to 5.0 (90 s) log CFU/g, with similar trends across marinade treatments. The numbers of pathogen survivors after reheating increased as populations on stored (7°C) product increased.

Significance: High levels of *L. monocytogenes* survivors after microwave oven reheating, especially after two days of storage, indicates that length of storage and reheating time need to be considered for safe consumption of leftover cooked chicken.

P3-158 Quadruplex PCR for Rapid Detection of Generic *Salmonella, Salmonella* Subspecies I, *S.* Typhimurium and *S.* Enteritidis in Layer Hen Housing Environments

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Introduction: FDA and National Poultry Improvement Program (NPIP) methods for isolation of *Salmonella* from environmental samples rely on selective enrichment and plating steps to screen for target cells against high levels of background microflora. Although these media are highly selective, some non-target organisms may still grow and yield false-positive reactions. These isolates must be tested further through biochemical testing, serotyping or generic PCR, resulting in added expense and delays in obtaining actionable data.

Purpose: To evaluate a novel quadruplex multiplex PCR (mPCR) for rapid detection and characterization of *Salmonella* isolates from layer hen environments.

Methods: A quadruplex mPCR for identification of generic *Salmonella*, *Salmonella* subspecies I, *S.* Typhimurium and *S.* Enteritidis was evaluated against a panel of *Salmonella* type strains and environmental isolates from layer hen housing environments. An additional set of environmental isolates that yielded false-positive reactions on XLT-4 or BGN agars, but were ultimately found not to be *Salmonella* based on serology were also evaluated. The identities of all strains used were determined independently via full-length 16S rDNA sequencing.

Results: Based on comparison of mPCR and 16S rDNA sequencing results, all *Salmonella* strains were correctly identified using the mPCR assay. Likewise, all false-positive environmental isolates were correctly identified as non-salmonellae via mPCR. These isolates were surprisingly diverse, belonging to various genera within the *Enterobacteriaceae*, including *Citrobacter*, *Enterobacter*, *Klebsiella*, *Escherichia*, *Providencia* and *Proteus*.

Significance: This approach provides an accurate and rapid method for quickly identifying *Salmonella* spp. among suspect isolates recovered from poultry production environments. Incorporation of this mPCR in an FDA/NPIP-based isolation workflow may speed the acquisition of actionable data on the presence of *Salmonella*, differentiate between generic *Salmonella, Salmonella* subspecies I, *S.* Typhimurium and *S.* Enteritidis, and eliminate wasteful downstream testing of false-positive non-*Salmonella* isolates.

P3-159 Thermal Inactivation of Avian Influenza Virus in Liquid Egg Products

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Introduction: Thirty-eight percent of the 200 million cases of shelled eggs produced per year in the U.S. are processed as liquid egg product. The pasteurization standards of egg products regulated by the U.S. Department of Agriculture (USDA) (9CFR Part 590.570) are based on a 5 $\log_{10}$ reduction of *Salmonella enterica* Enteritidis. The pasteurization standard for sugared egg yolk is 63.3°C for 3.5 min or 62.2°C for 6.2 min; for fortified egg yolk (32% solid) is 62.2°C for 2.0 min; plain egg yolk for 60°C for 6.2 min or 61.1°C for 3.5 min; and 10%-salted egg yolk at 62.2°C for 6.2 min or 63.3°C for 3.5 min.

Purpose: The objective of this study is to develop predictive models for the heat inactivation of avian influenza in liquid egg products.

Methods: Survival curves were generated to determine D and Z_D values of low pathogenic avian influenza A/ck/NY/13142/94 (H7N2) artificially inoculated into fortified, sugared and plain egg products at 106.3 EID50/ml then heat treated at various temperatures for 0, 1, 2, 3, 4, 6 min and 8 min.

Results: The D-values for 10% sugared egg product resulted in D56, D57, D58, D59, D60, D61, D62.2, and D63.3 of 0.63, 0.52, 0.47, 0.29, 0.26, 0.24, 0.23, and 0.19 min, respectively, and a z_D value was 1.2°C. For fortified egg product the D57, D58, D59, D60, D61.1, and D62 values were 0.68, 0.44, 0.40, 0.36, 0.13, and 0.13 min, respectively, and a Z_D value of 0.7°C. For plain egg yolk (38 % solid) the D57, D58, D59, D60, D61.1, and D62 values were 0.62, 0.61, 0.60, 0.60, 0.59, and 0.48 min, respectively, and a Z_D value of 0.02°C. For salted egg yolk product the D58, D59, D60, D61, D62.2, and D63.3 values were 0.69, 0.69, 0.68, 0.67, 0.37, and 0.3 min respectively, and a Z_D value of 0.03°C.

Significance: The data demonstrate that LPAI would not survive the pasteurization processes for liquid egg products.

P3-160 Thermal Inactivation Kinetics of *Salmonella* Enteritidis and Oranienberg in Commercially-acquired 10%-Salted Liquid Whole Egg

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Introduction: Liquid egg pasteurization requirements are based on time/temperature combinations in the Code of Federal Regulations, Title 9, Ch. III, Sec. 590.570 from data acquired prior to 1970. These guidelines are being reevaluated in light of recent risk assessments.

Purpose: The goal of this study was to determine the inactivation kinetics of thermally-resistant *Salmonella* in commercially-acquired 10%-salted liquid whole egg (LWE).

Methods: Heat-resistant *Salmonella* Enteritidis and Oranienburg were grown in Tryptic Soy Broth at 37°C, composited and added to 10%-salted LWE and mixed, resulting in final populations of ca. 5.7-7.8 log CFU/ml. Inoculated egg was injected into glass capillary tubes, flame-sealed and heated in a water bath at 60, 62.2, 63.3 64.3 or 66°C. Contents were surface plated, incubated at 37°C for 24 h, and colonies were enumerated. Random presumptive-positive colonies were confirmed by selective differential plating and serological agglutination.

Results: Survival curves were not log-linear (log levels versus time), but decreased rapidly, and after initial periods became linear. Asymptotic decimal reduction values at each temperature were calculated from survivor curves with a minimum inactivation of 5.0 log CFU/ml. The asymptotic thermal D-values for salted LWE were 3.05, 1.70, 1.25, 1.00 and 0.63 min at 60, 62.2, 63.3 64.3 or 66°C, respectively. The calculated thermal z-value was 8.8°C. A model that predicts lethality for given times and temperatures was developed. The D- and z-values reported in our study are similar to those reported in the literature and confirm that the current pasteurization requirements for 10%-salted LWE of minimum temperatures, times of 63.3°C for 3.5 min, or 62.2°C for 6.2 min, are not sufficient to inactivate 7 log CFU/ml of *Salmonella* and are estimated to provide ca. 4 –5 log CFU/ml of destruction.

Significance: This model will assist the USDA-FSIS in issuing pasteurization performance standards and provide industry guidance for designing pasteurization processes that will ensure safe product.

P3-161 The Heat Destruction of *Salmonella* Enteritidis in Liquid Egg White as a Function of Heat Treatment, Temperature and Heating Rate

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Introduction: Some microbes such as *Salmonella* show an increasing resistance after heat shock. Such a heat shock could be the heating of refrigerated liquid eggs to the temperature of long time (6–24 h) heat treatment.

Purpose: The aim of our work was to investigate the extent of heat resistance changes of *Salmonella* Enteritidis in liquid egg products during the heating from 4°C to 50–55°C in 5-60 minutes.

Methods: The liquid egg white (pH=8.9 ± 0.1) was purchased from a Hungarian egg processing factory. Prior to the experiment the liquid egg white samples inoculated with *Salmonella* Enteritidis were placed into sterile, adjustable-temperature thermostate. The samples were stirred during the experiment. The heating rate was linear: 0.76; 2; 5; 8 and 9.24°C/min to the final temperature 48.96; 50.0; 52.0; 55 and 56.04°C. The samples were held at the final temperature for 30 and the changes of the colony counts were determined.

Results: We used Central Composite Rotatable Design (CCRD) in our experiment and Response Surface Method (RSM) was used to evaluate the data. Our results pointed out that beside the temperature of heat treatment the heating rate have also an effect on the heat destruction of *Salmonella* Enteritidis. In case of heating rate 9.24°C/min the D52,5-value was 2.32 min, however at heating rate 0.76°C/min the D52,5-value was 19.23 min.

Significance: We can conclude that the heating rate and the holding temperature have an effect on the heat resistance of *Salmonella* Enteritidis in liquid egg white. This should be considered particularly in case of technologies where the refrigerated liquid egg white is heated to heat treatment temperature for a relatively long time.

P3-162 Consumer Confidence in Food Related Practices: Is It a Valid Measure of Food Safety?

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Introduction: Many consumers may link the causes of foodborne illness to the food industry while overlooking their own cooking practices. Most feel it is unlikely they will contract a foodborne illness within their own homes, despite the fact that many foodborne illnesses occur as the result of cross contamination, improper hand washing, or poor temperature control.

Purpose: The purpose of this study was to investigate whether consumers who were confident that they exhibited safe food-related practices actually did so.

Methods: Consumers from 150 households in Middle Tennessee participated in this two part study. In each household, the person mainly responsible for food purchase, storage, and preparation was interviewed concerning their food safety practices. Following the interview, 102 of the consumers agreed to participate in an observational study where they prepared a meatloaf in their kitchen. All the ingredients were provided; consumers were asked to store them where they would normally place them following purchase. Researchers completed a food preparation checklist while observing the participants, from which risky behaviors were identified.

Results: The majority (82%) believed they were doing all they could to keep food safe at home. However, only 77% of them reported having a refrigerator thermometer, only 33% had a specific cutting board for fruits and vegetables, and only 4% reported using a food thermometer the last time they cooked chicken parts. When asked to store the ingredients, only 24% of the participants in part two of the study stored the raw meat in the correct location in the refrigerator. While 88% of the participants washed their hands before food preparation, only 57% washed their hands after touching the raw meat package. Of those that did wash their hands, only 26% washed for longer than 20 seconds.

Significance: This study demonstrated that consumers, even those who feel they are doing everything they can to keep their food safe at home, are putting themselves at risk for a foodborne illness. Innovative techniques are needed to educate consumers in safe food-handling practices.